DRAFT
TOXICOLOGICAL PROFILE FOR
MUSTARD GAS

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

September 2001

DRAFT FOR PUBLIC COMMENT

DISCLAIMER

The use of company or product name(s) is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry.

UPDATE STATEMENT

Toxicological profiles are revised and republished as necessary, but no less than once every three years. For information regarding the update status of previously released profiles, contact ATSDR at:

Agency for Toxic Substances and Disease Registry
Division of Toxicology/Toxicology Information Branch
1600 Clifton Road NE, E-29
Atlanta, Georgia 30333

FOREWORD

This toxicological profile is prepared in accordance with guidelines developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the hazardous substance described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a hazardous substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protection of public health are identified by ATSDR and EPA.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;

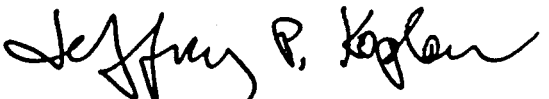
(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

The toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed ATSDR to prepare toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. The availability of the revised priority list of 275 hazardous substances was announced in the *Federal Register* on October 21, 1999 (64 FR 56792). For prior versions of the list of substances, see *Federal Register* notices dated April 17, 1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26, 1989 (54 FR 43619); October 17, 1990 (55 FR 42067); October 17, 1991 (56 FR 52166); October 28, 1992 (57 FR 48801); February 28, 1994 (59 FR 9486); April 29, 1996 (61 FR 18744); and November 17, 1997 (62 FR 61332). Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staff of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and is being made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.



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Disease Registry

QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances will find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Public Health Statement: The Public Health Statement can be a useful tool for educating patients about possible exposure to a hazardous substance. It explains a substance's relevant toxicologic properties in a nontechnical, question-and-answer format, and it includes a review of the general health effects observed following exposure.

Chapter 2: Relevance to Public Health: The Relevance to Public Health Section evaluates, interprets, and assesses the significance of toxicity data to human health.

Chapter 3: Health Effects: Specific health effects of a given hazardous compound are reported by *type of health effect* (death, systemic, immunologic, reproductive), by *route of exposure*, and by *length of exposure* (acute, intermediate, and chronic). In addition, both human and animal studies are reported in this section.

NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting. Please refer to the Public Health Statement to identify general health effects observed following exposure.

Pediatrics: Four new sections have been added to each Toxicological Profile to address child health issues:

Section 1.6	**How Can (Chemical X) Affect Children?**
Section 1.7	**How Can Families Reduce the Risk of Exposure to (Chemical X)?**
Section 3.7	**Children's Susceptibility**
Section 6.6	**Exposures of Children**

Other Sections of Interest:

Section 3.8	**Biomarkers of Exposure and Effect**
Section 3.11	**Methods for Reducing Toxic Effects**

ATSDR Information Center

Phone: 1-888-42-ATSDR or (404) 498-0110 ***Fax:*** (404) 498-0057
E-mail: atsdric@cdc.gov ***Internet:*** http://www.atsdr.cdc.gov

The following additional material can be ordered through the ATSDR Information Center:

Case Studies in Environmental Medicine: Taking an Exposure History—The importance of taking an exposure history and how to conduct one are described, and an example of a thorough exposure history is provided. Other case studies of interest include *Reproductive and Developmental Hazards; Skin Lesions and Environmental Exposures; Cholinesterase-Inhibiting Pesticide*

Toxicity; and numerous chemical-specific case studies.

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident. Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposures*—is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets (ToxFAQs) provide answers to frequently asked questions about toxic substances.

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. *Contact:* NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. *Contact:* NIOSH, 200 Independence Avenue, SW, Washington, DC 20201 • Phone: 800-356-4674 or NIOSH Technical Information Branch, Robert A. Taft Laboratory, Mailstop C-19, 4676 Columbia Parkway, Cincinnati, OH 45226-1998 • Phone: 800-35-NIOSH.

*The National Institute of Environmental Health Sciences (*NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. *Contact:* NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212.

Referrals

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. *Contact:* AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. *Contact:* ACOEM, 55 West Seegers Road, Arlington Heights, IL 60005 • Phone: 847-818-1800 • FAX: 847-818-9266.

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.

2. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific minimal risk levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.

3. Data Needs Review. The Research Implementation Branch reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.

PEER REVIEW

A peer review panel was assembled for mustard gas. The panel consisted of the following members:

1. Dr. Mohammed Mustafa, Associate Professor, Division of Environmental and Occupational Sciences, University of California, Los Angeles, California.

2. Mr. Bruce Jacobs, Chief Health Scientist, General Physics Corporation, Columbia, Maryland.

3. Dr. Raymond Smith, Instructor, Department of Pathology and Microbiology, University of Nebraska, Omaha, Nebraska.

These experts collectively have knowledge of mustard gas' physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

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1. PUBLIC HEALTH STATEMENT

This public health statement tells you about mustard gas and the effects of exposure.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites make up the National Priorities List (NPL) and are the sites targeted for long-term federal cleanup activities. Mustard gas has been found in at least 3 of the 1,585 current or former NPL sites. However, the total number of NPL sites evaluated for mustard gas is not known. As more sites are evaluated, the sites at which mustard gas is found may increase. This information is important because exposure to mustard gas may harm you and because these sites may be sources of exposure.

When a substance is released from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment. This release does not always lead to exposure. You are exposed to a substance only when you come in contact with it. You may be exposed by breathing, eating, or drinking the substance, or by skin contact.

If you are exposed to mustard gas, many factors determine whether you'll be harmed. These factors include the dose (how much), the duration (how long), and how you come in contact with it. You must also consider the other chemicals you're exposed to and your age, sex, diet, family traits, lifestyle, and state of health.

1.1 WHAT IS MUSTARD GAS?

The term mustard gas refers to several chemicals. In its most common sense, it means sulfur mustard, which is the chemical that is stored at Army facilities. Mustard gas does not behave as a gas under ordinary conditions. The commonly used term mustard gas can be confusing, since the compound is stored as a liquid and is not likely to change into a gas immediately if it is released at ordinary temperatures. As a liquid, it is colorless when pure and it is brown when mixed with other chemicals. It is odorless when pure, but can have a slight garlic smell when

mixed with other chemicals. It dissolves easily in fats, oils, alcohol, and gasoline. Mustard gas dissolves slowly in unstirred water, but within minutes in stirred water. When it does dissolve, it turns into different chemicals. It was made to be used in chemical warfare and was used as early as World War I and as late as the Iran-Iraq War in 1980–1988. It is not used in the United States, except for laboratory testing of health effects and antidotes. More information on the physical and chemical properties of mustard gas can be found in Chapters 4 and 5.

1.2 WHAT HAPPENS TO MUSTARD GAS WHEN IT ENTERS THE ENVIRONMENT?

Mustard gas is not found naturally in the environment in any amount. If mustard gas is accidentally spilled at an Army base where it is stored, it could be released into the environment. Currently, all of the mustard gas at these Army bases is being destroyed by burning. The law requires that the Department of Defense destroy all mustard gas by 2004. Once all of the mustard gas is destroyed, it will no longer be dangerous. If mustard gas is put on soil, it will remain there for at least a day, but may remain up to several days until it disappears. The time it takes for mustard gas to disappear from soil depends on how hot it is outside and how strongly the wind is blowing. If it is hot and the wind is strong, then mustard gas will disappear faster. When mustard gas disappears from soil, it becomes a gas or changes into other compounds if the soil is wet. However, if mustard gas is buried underground, it may not disappear for several years. Mustard gas will not move through soil to underground water. If mustard gas is put in water, it dissolves within minutes if the water is stirred, and slowly if is not. When it does dissolve, it changes to other compounds. The time necessary for a quantity of mustard gas that is dissolved in water to decrease by half is about 2 minutes at 40 EC (104EF). If large amounts of mustard gas are spilled into water, most of the mustard gas will change to other compounds very slowly or not at all. If mustard gas is released into air, it will react with components in the air to form other compounds. The time necessary for a quantity of mustard gas in air to decrease by half is about 2 days at 25 EC (77EF). Because mustard gas changes to other chemicals in the environment, it will not concentrate in plants or animals. For more information on what happens when mustard gas enters the environment, see Chapter 6.

1.3 HOW MIGHT I BE EXPOSED TO MUSTARD GAS?

Mustard gas is not currently being produced in the United States. The only possibility of exposure of the general public is through accidental release from the Army bases where it is stored. Storage areas are heavily guarded, and storage buildings are sealed. People who work at these Army bases are more likely to be exposed. Mixed in water, mustard gas changes its form within minutes, so it is very unlikely that you would ever drink it. Any possibility of exposure of the general population by way of water (drinking, cooking, bathing, swimming) is therefore very small. Mustard gas does not occur naturally, and, therefore, there are no background levels in the soil, air, water, or food. If it is accidentally released, it will stay in the air or on the ground for 1–3 days. For more information on possible exposures, see Chapter 6.

1.4 HOW CAN MUSTARD GAS ENTER AND LEAVE MY BODY?

Mustard gas can enter your body easily and quickly if you breathe the gas vapors or if you get it on your skin. It can easily pass through your clothing to get onto your skin. It is possible that you could breathe mustard gas or get it on your skin at hazardous waste sites that contain this material. Mustard gas changes into other chemicals in your body, and these chemicals mostly leave your body in the urine within a few weeks. For more information, see Chapter 3.

1.5 HOW CAN MUSTARD GAS AFFECT MY HEALTH?

One way to see if a chemical will hurt people is to learn how the chemical is absorbed, used, and released by the body; for some chemicals, animal testing may be necessary. Animal testing may also be used to identify health effects such as cancer or birth defects. Without laboratory animals, scientists would lose a basic method to get information needed to make wise decisions to protect public health. Scientists have the responsibility to treat research animals with care and compassion. Laws today protect the welfare of research animals, and scientists must comply with strict animal care guidelines.

Mustard gas burns your skin and causes blisters within a few days. The parts of your body that are sweaty are the most likely to be harmed. Mustard gas makes your eyes burn, your eyelids swell, and causes you to blink a lot. If you breathe it, mustard gas can cause coughing, bronchitis, and long-term respiratory disease. Mustard gas may affect reproduction. Some men exposed to mustard gas during war have reported decreased sexual drive and have had lower sperm counts. The Department of Health and Human Services has determined that mustard gas is a known carcinogen. The International Agency for Research on Cancer has also determined that mustard gas is carcinogenic to humans. It can cause cancer in your airways and lungs later in life. If you are exposed to a very large amount of mustard gas, you can eventually die from it. Some of the chemicals that are formed when mustard gas is burned or spilled into water can also be irritating to the skin.

1.6 HOW CAN MUSTARD GAS AFFECT CHILDREN?

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans.

Mustard gas causes the eyes and skin of children to burn similarly to adults; however, the burns are more severe in children. Blisters appear sooner, as early as 4 hours after mustard gas contact with skin. Coughing and vomiting are early symptoms of exposure to mustard gas in children. Mustard gas vapors are heavier than air and since young children are closer to the ground or floor because of their height, they may be exposed to more mustard gas vapors than adults during accidental exposures. Mustard gas may cause birth defects or affect the development of children. An increased incidence of birth defects has been reported among newborn babies of mustard gas victims exposed during war. Studies in animals also indicate that mustard gas may affect development. It is not known if mustard gas can cross the placenta or be passed to infants in breast milk.

1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO MUSTARD GAS?

If your doctor finds that you have been exposed to significant amounts of mustard gas, ask whether your children might also be exposed. Your doctor might need to ask your state health department to investigate.

The risk of exposure to mustard gas may be significant only for those who live or work near Army bases and facilities that store it. Mustard gas is currently being destroyed at these facilities and thus, the risk of exposure due to accidents is decreasing.

1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO MUSTARD GAS?

There are effective medical tests to determine if you have been exposed to mustard gas. Mustard gas or some of the chemicals that it makes in your body can be found by testing your blood or urine. For more information, see Chapters 3 and 7.

1.9 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

The federal government develops regulations and recommendations to protect public health. Regulations can be enforced by law. Federal agencies that develop regulations for toxic substances include the Environmental Protection Agency (EPA), the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA). Recommendations provide valuable guidelines to protect public health but cannot be enforced by law. Federal organizations that develop recommendations for toxic substances include the Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH).

Regulations and recommendations can be expressed in not-to-exceed levels in air, water, soil, or food that are usually based on levels that affect animal, and these levels are then adjusted to help protect people. Sometimes these not-to-exceed levels differ among federal organizations because of different exposure times (an 8-hour workday or a 24-hour day), the use of different animal studies, or other factors.

Recommendations and regulations are periodically updated as more information becomes available. For the most current information, check with the federal agency or organization that provides it. Some regulations and recommendations for mustard gas include the following:

The federal government considers mustard gas an extremely hazardous substance. The federal government has recommended a maximum concentration in air to which the general public should be exposed. This concentration is 0.0001 milligrams per cubic meter of air, averaged over 3 days. Stored quantities of 500 pounds or more must be reported to the State Emergency Response Commission, the fire department, and the Local Emergency Planning Committee. Spills of over 1 pound must be reported to the National Response Center. For more information, see Chapter 8.

1.10 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns, please contact your community or state health or environmental quality department or

Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road NE, Mailstop E-29
Atlanta, GA 30333

* Information line and technical assistance

Phone: 1-888-42-ATSDR (1-888-422-8737)
Fax: 1-404-498-0057

ATSDR can also tell you the location of occupational and environmental health clinics. These clinics specialize in recognizing, evaluating, and treating illnesses resulting from exposure to hazardous substances.

* To order toxicological profiles, contact

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Phone: 1-800-553-6847 or 1-703-605-6000

2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO MUSTARD GAS IN THE UNITED STATES

Mustard gas does not naturally occur, and, therefore, there are no background levels in the soil, air, water, or food. Mustard gas is a chemical warfare agent. Its use has been confirmed in World War I and in the Iran-Iraq War, and there are reports of mustard gas being utilized in other conflicts. The production of mustard gas in the United States was discontinued in the mid-1950s. Since mustard gas is no longer produced or used commercially in the United States, occupational and general population exposures are expected to be low. The U.S. stockpile of mustard gas is currently stored at seven sites in the continental United States and one site located on Johnston Island in the Pacific Ocean. If mustard gas is accidentally released into the air, the primary routes of exposure would be inhalation or contact with eyes and skin. Mustard gas has been found in at least 3 of the 1,585 current or former NPL sites. At hazardous waste sites, exposure to mustard gas is also possible by dermal contact with contaminated soil or containers. Children are expected to be exposed to mustard gas by the same routes as adults.

2.2 SUMMARY OF HEALTH EFFECTS

Numerous reports of combat exposures to mustard gas provide strong evidence of the toxic potential of mustard gas. Additional information on the health effects of mustard gas is available from studies of mustard gas factory workers and mustard gas testing of volunteers. As summarized below and detailed in Chapter 3, effects that have been associated with exposure to mustard gas in humans and/or animals include ocular and dermal injury, respiratory tract irritation, reproductive and developmental toxicity, gastrointestinal effects, hormone alterations, hematological and lymphoreticular effects, and cancer. Combat mustard gas exposure levels have not been quantified, and blast effects may be present concurrently. Studies of mustard gas workers are complicated by possible concurrent exposure to other toxic agents because factories generally produced multiple chemical warfare agents. The lack of follow-up health assessments of human subjects in gas chamber and field tests limits the assessment of long-term health consequences. Therefore, available human data are not appropriate for quantitative risk assessments. Animal studies have shown that mustard gas induces similar toxic effects in animals and humans, with the exception of blistering of animals that have fur. However, most animal studies have been conducted with mustard gas administered by oral or intravenous routes.

Direct Contact Effects. Data from soldiers and civilians exposed during combat, mustard gas factory workers, mustard gas testing volunteers, and accidental exposures provide ample evidence of the toxic potential of mustard gas to tissues coming into direct contact with mustard gas. Mustard gas exposure results in eye irritation and burning of the skin, which begins several hours after exposure. The severity of cutaneous injury is dose- and temperature-dependent and is directly related to the mustard gas alkylation levels in skin. It is likely that direct contact with other tissues would have these same dependencies. Due to the aqueous nature and accessibility, the eyes are more sensitive to mustard gas than the skin or respiratory tract. The damage may vary from mild conjunctivitis to severe corneal involvement with dense opacification, ulceration, and vascularization. Delayed ocular reactions, manifested as delayed relapsing keratitis, may also occur. Early respiratory effects include shortness of breath, a burning sensation of the vocal cords, and hemorrhagic inflammation of the tracheobronchial mucosa accompanied by severe erosions or membranous lesions. Children appear to be more sensitive to the irritant effects of mustard gas with manifestations of exposure occurring as early as 4 hours after exposure, whereas effects in adults are generally delayed by at least 8 hours. In children, cough was the first respiratory symptom. Breathing pattern alterations and erosions of the airway mucosa have also been reported in animals. Prolonged inhalation exposure can result in chronic bronchitis or cancer of the respiratory passages and lungs. Stomach irritation and inflammation and bleeding of the gastric mucosa were reported in victims of combat exposure where at least small amounts were likely ingested. Similar effects have been observed in animal studies.

Reproductive Effects. While the routes of exposure differ, animal reproductive effects data support the long-term effects reported in humans. In a follow-up study of men who were injured by mustard gas during the Iran-Iraq War, reduced sperm counts were reported. An increased rate of fetal deaths and an altered sex ratio were reported in progenies of Iranian survivors of chemical attacks that included mustard gas. An increase in fetal mortality also occurred in an animal study subsequent to the mating of orally exposed male and unexposed female rats. Altered sex ratios have been reported in a study in rats orally exposed to mustard gas. Abnormal sperms shapes were observed in rats exposed to mustard gas by the oral route. The reproductive effects appear to be male dominant as no changes were seen in the number of live fetuses or resorptions in a study of pregnant rats exposed to mustard gas in the air.

Developmental Effects. An increased incidence of congenital malformations (skeletal and muscle abnormalities, limb defects, anacephaly, hydrocephaly, microcephaly, cleft lip and palate, deafness, blindness, and mental retardation) was reported among offspring of Iranian mustard gas victims. The study could not distinguish whether the observed defects were caused only by exposure to chemical agents or by other environmental factors as well. In animal fetuses, incidences of reduced ossification and reduced body weight have occurred when the mother was exposed to mustard gas doses that produced maternal toxicity.

Cancer. There is sufficient evidence that mustard gas is carcinogenic to humans. Epidemiological studies on World War I victims exposed to mustard gas revealed an association between respiratory exposure and the risk of developing lung cancer. Factory workers exposed to mustard gas for a number of years have been shown to develop respiratory cancer. Although most human studies have found an association between mustard gas exposure and respiratory cancer, some studies have not found a significant relationship, possibly due to lower exposure levels. It is also documented that occupational dermal exposure to mustard gas produces Bowen's diseases (precancerous dermatitis) in humans. Two animal studies, of low predictive quality due to species strain tendency to develop lung tumors, insufficient animals, and inadequate doses, have also shown increases in tumors from mustard gas exposure in the air. Subcutaneous, intramuscular, and intravenous injections of mustard gas into mice have also produced increased tumors at the site of the injection, in the mammary glands, or in the lungs.

2.3 MINIMAL RISK LEVELS (MRLs)

The details regarding calculations of the MRLs for mustard gas are described in Appendix A.

Inhalation MRLs

- An MRL of 0.0002 mg/m^3 has been derived for acute-duration inhalation exposure (14 days or less) to mustard gas.

There are numerous reports of exposure to mustard gas during combat and in mustard gas factories; however, the exposure levels or durations were not quantified, and therefore, these data are inadequate for deriving dose-response relationships. The acute-duration inhalation MRL was based on a lowest-observed-adverse-effect level (LOAEL) of 21.3 mg/m^3 for respiratory effects in mice that were exposed

for 1 hour. The LOAEL was duration-adjusted to a 24-hour exposure period and dosimetrically adjusted for humans, and an uncertainty factor of 300 [10 for use of a LOAEL, 3 for extrapolation from animals to humans using a dosimetric adjustment, and 10 for human variability] and a modifying factor of 3 [for proximity to serious effects (28% body weight loss at 16.9 mg/m^3)] were applied to the LOAEL to derive the MRL (see Appendix A for details). Groups of female Swiss albino mice were administered 8.5, 16.9, 21.3, 26.8, 42.3, or 84.7 mg/m^3 of mustard gas by inhalation for 1 hour. At all mustard gas concentrations, mice exhibited reversible sensory irritation, characterized by a pause between inspiration and expiration, and the respiratory frequency decreased to a slower steady state after 30 minutes of exposure. While sensory irritation was reversible (normal respiration pattern was recovered after inhalation exposure was terminated), delayed effects of mustard gas were indicated by a significant reduction in respiratory frequency beginning 48 hours after exposure at concentrations of $21.3 mg/m^3. The depression in respiratory frequency following exposure was related to both concentration and postexposure time. Airflow limitation, evidenced by the decreased respiratory rate, is thought to occur due to the effect of mustard gas on the tracheal secretory cells. In addition to the respiratory effects, significant ($25%) reductions in body weight occurred at $16.9 mg/m^3 at 7 days postexposure; however, the magnitude of the weight reduction at 16.9 mg/m^3 (28%) was greater than that observed in two other studies in mice and guinea pigs in which weight reductions of only 14% were reported at 85 and 125 mg/m^3, respectively, at similar postexposure times. ATSDR considers the respiratory system to be the critical target for acute toxic effects of mustard gas, and the concentration of 21.3 mg/m^3 to be a LOAEL for delayed respiratory effects beginning 48 hours after exposure, for derivation of an acute-duration inhalation MRL.

There is ample evidence of mustard gas-induced respiratory effects in humans following acute and chronic exposures. Soldiers reported shortness of breath and early respiratory manifestations including hemorrhagic inflammation of the tracheobronchial mucosa accompanied by severe erosions or membranous lesions. Some exposed soldiers became temporarily aphonic due to an acid-like burning sensation of the vocal cords. Coughing was the first respiratory symptom in children. Secondary complications consisted of extensive stenosis of sections or the entire tracheobronchial tree, suppurative bronchitis, and chronic respiratory infections. Scars, ulcers, strictures, and nonspecific fibrous granulation developed in central airways after a delay of up to 15 months. Progressive deterioration of lung compliance and gas exchange with resulting hypoxemia and hypercapnia were common with injury. Chronic respiratory complaints included shortness of breath, chest tightness, cough, sneezing, rhinorrhea, and sore throats. Long-term or delayed effects included central airway stenosis, bronchiectasis, bronchiolitis, and bronchitis.

Other studies show that factory workers who were apparently exposed to mustard gas for a few years developed acute and chronic respiratory effects. Factory workers in Britain who were exposed to mustard gas also showed increased deaths due to acute and chronic nonmalignant respiratory disease, including influenza and pneumonia. Workers in a Japanese poison gas factory were more likely to have chronic bronchitis, chronic cough, and decreased respiratory volume than nonexposed persons. A significantly increased incidence of mortality from pneumonia was reported among 428 former workers of a mustard gas manufacturing facility.

Short-term respiratory effects similar to those described in humans have been reported in experimental animals. Microscopic changes of the airways and lungs were observed in rabbits and dogs exposed to mustard gas vapor. The major pathological changes were in the nasal passages, pharynx, larynx, and upper portion of the respiratory tract. In animals with severe injuries that survived beyond a few days, the lesions incurred secondary infections, as in humans, leading to bronchopneunomia, which apparently was the cause of death in many cases. Subsequent to acute (#1 hour) inhalation exposure, a decrease in respiratory rate that lasted for up to 7 days after exposure was reported in mice and guinea pigs.

- MRLs for intermediate- (15–364 days) and chronic-duration (364 days or more) inhalation exposure to mustard gas have not been derived because quantitative data were not available to determine no-observed-adverse-effect levels (NOAELs) or LOAELs.

Oral MRLs

- An MRL of 0.0005 mg/kg/day (0.5 µg/kg/day) has been derived for acute-duration oral exposure (14 days or less) to mustard gas.

The acute-duration oral MRL was based on a LOAEL of 0.5 mg/kg/day for inflamed mesenteric lymph nodes in rat dams administered mustard gas by intragastric intubation. The dose is also a LOAEL for reduced ossification in the fetuses. An uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability) was applied to the LOAEL to derive the MRL. There were no treatment-related deaths in groups of 25–27 mated Sprague-Dawley female rats (10–11 weeks old) that were dosed acutely on gestation days 6–15 (10 days) with 0, 0.5, 1.0, or 2.0 mg/kg/day mustard gas (95.9–96.1% purity) in sesame oil in a teratology study. Significant incidences of inflamed mesenteric lymph nodes in dams and reduced ossification in fetuses occurred with mustard gas doses $0.5 mg/kg/day (see Appendix A for additional details).

There is some evidence for mustard gas-induced lymph system effects in humans. Lymph node discoloration and spleen pathology were found in autopsies of mustard gas victims. Additional studies in animals also indicate mustard gas-induced damage to the lymph system. Incidences of inflamed mesenteric lymph nodes occurred at $0.4 mg/kg/day in dose-range experiments and another lymphoretic effect, enlarged Peyer's patches, was observed in rabbits at 0.5 mg/kg/day in a range-finding study and at 0.4 mg/kg/day in a teratology study (incidence data not reported).

- An MRL of 0.00002 mg/kg/day (0.02 µg/kg/day) has been derived for intermediate-duration oral exposure (15–364 days) to mustard gas.

The intermediate-duration oral MRL was based on a dose of 0.03 mg/kg/day for gastrointestinal effects in rats administered mustard gas by intragastric intubation. The corresponding time-weighted average LOAEL used in the MRL derivation was 0.02 mg/kg/day (see Appendix A for details). An uncertainty factor of 1,000 was applied to the LOAEL to derive the MRL. In a two-generation reproduction study, groups of 8-week-old Sprague-Dawley rats (27 female and 20 males/group/generation) were dosed with 0, 0.03, 0.1, or 0.4 mg/kg/day mustard gas (97.3% purity) in sesame oil. Male and female rats were dosed 5 days/week for 13 weeks before mating and during a 2-week mating period. Males were dosed 5 days/week during the 21-day gestation period. Females were dosed daily (7 days/week) throughout the 21-day gestation period and 4–5 days/week during the 21-day lactation period. Mated males were sacrificed at the birth of their pups, and dams were sacrificed when their pups were weaned. Male and female F1 pups were treated with mustard gas until they were mated and the females became pregnant and gave birth. The dosing of F1 dams continued until pup weaning, at which time, the study was terminated. Significant dose-related incidence and severity of hyperplasia of the squamous epithelium of the forestomach occurred in both sexes with mustard gas doses $0.03 mg/kg/day (see Appendix A for additional details).

In support of the critical effect, gastrointestinal effects have been reported in humans following combat exposure to mustard gas, in mustard gas testing volunteers, and in mustard gas factory workers. Gastrointestinal effects (edema, hemorrhage or sloughing of the mucosa, and ulceration) were also observed in rabbits following 14-day exposures at $0.4 mg/kg/day, in rats following 10-day exposures at $2.0 mg/kg/day, and in rats following 13-week exposures at $0.1 mg/kg/day.

- An MRL for chronic-duration oral exposure (364 days or more) to mustard gas has not been derived because quantitative data were not available to determine NOAELs or LOAELs.

2. RELEVANCE TO PUBLIC HEALTH

No studies were located regarding the toxicity of mustard gas in humans or animals following chronic oral exposure.

3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of mustard gas. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

The term mustard gas is used to refer to a variety of compounds. In its most common sense, it refers to sulfur mustard (bis[2-chloroethyl]sulfide). This is the compound discussed in this profile. The term mustard gas has also been occasionally applied to nitrogen mustards, which have not been reported at NPL sites and are not evaluated in this profile. Mustard gas is a clear, colorless, oily liquid. As a warfare or terrorist agent, mustard gas may have been dispersed by spraying or by explosive blasts producing a vapor, aerosol, and/or liquid droplets. Persons involved in the manufacture, transport, or disposal of mustard gas may also be exposed occupationally. Mustard gas corrodes and weakens containers and may be dispersed in water, cleaning solvents, or by evaporation in air. Mustard gas is slightly soluble in water, but both the liquid and vapor forms are readily soluble in alcohol, gasoline, kerosene, oils, fats, and organic solvents. Mustard gas is environmentally persistent. Evaporation in air increases with increasing temperatures, but at temperature below 14 EC, it freezes and remains in active form. Both liquid and vapor forms readily penetrate ordinary clothing. The effects of mustard gas poisoning may be local, systemic, or both, depending on environmental conditions, exposed organs, and extent and duration of exposure. Because of the high lipid solubility, mustard gas quickly penetrates the lipid cell membrane. Although mustard gas may be lethal, it is more likely to cause extensive incapacitating injuries to the eyes, skin, and respiratory tract of exposed persons. Alkylation reactions of mustard gas with tissue are rapid and irreversible; however, cutananeous lesions do not become apparent for one to several hours after exposure. Burns caused by mustard gas are typically severe and require long healing periods.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for mustard gas. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

While mustard gas is described as smelling like mustard, horseradish, garlic, or onions, it can be difficult to smell and may not be recognized by the general population. Due to the delayed symptoms and difficulties associated with detection by smell, individuals may not know that they are being exposed, and consequently, appropriate actions may not be taken. The odor threshold for mustard gas is 0.6 mg/m^3 (0.0006 mg/L) (SBCCOM 1999). In humans, an ICt_{50} (estimated concentration-exposure time period product incapacitating to 50% of exposed individuals) for inhalation exposure is 1,500 mg-minute/m^3 (SBCCOM 1999).

3.2.1.1 Death

Human deaths associated with mustard gas exposure occurred during World War I (an estimated 28,000 deaths), during the Italian attack on the Ethiopians in 1936, and during the Iran-Iraq War in 1980–1988. Battlefield air concentrations of mustard gas vapor during attacks in World War I were estimated in the range 19–33 mg/m^3 (Solberg et al. 1997). During chemical warfare, exposure to mustard gas generally occurred by multiple routes. Mustard gas can cause death in several ways, and with multiple routes, it is often difficult to determine the relative importance of local and systemic effects in causing death. Death is often accompanied by heavy and painful coughing, vomiting, burning eyes, and shock. Deaths have occurred immediately following exposure in the battlefield, most likely due to acute chemical-induced pulmonary edema (Freitag et al. 1991). Mustard gas has caused death within a few hours of exposure by inducing shock in victims of the World War II Bari Harbor incident and in civilians who accidentally recovered unspent World War I mustard gas shells (Alexander 1947; Papirmeister et al. 1991). Deaths beyond the second day after the Bari incident were attributed to decreased leukocyte counts, which reached levels below 100 cell/cm^3 (Dacre and Goldman 1996). While mustard gas was not used during World War II, cargo vessels in the harbor of Bari, Italy, carrying mustard gas and explosive munitions were bombed by German planes. In the resulting explosion, mustard gas was released in to the air and water, exposing survivors to mustard gas vapor and to a mixture of mustard gas in oil. Deaths, which occurred in 1–4% of the soldiers exposed during World War I, were largely due to secondary respiratory infections. Accidental death of a family of two adults and two children occurred in 1919 in Salaise, France after exposure to mustard gas, which evaporated from a leaking can of mustard gas-contaminated alcohol that was being stored in the house (Dacre and Goldman 1996).

One death among 14 children (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years) admitted to a hospital in Iran 18–24 hours following exposure to mustard gas from air bombs during the Iran-Iraq War was reported (Momeni and Aminjavaheri 1994). The 13-month-old girl developed pancytopenia and respiratory failure, and died 8 days after exposure. Deaths have also occurred from delayed responses (DOA 1988; Lohs 1975; Somani and Babu 1989). Further information on delayed death due to inhalation of mustard gas by humans is discussed below in the sections on respiratory effects in Section 3.2.1.2 and on cancer in Section 3.2.1.7. Lethal doses for humans have been reported, but these data cannot be considered to be reliable because all elements of exposure were not clearly described (Frank 1967; Rosenblatt et al. 1975).

Rabbits and monkeys that had undergone tracheal cannulation were exposed to nominal chamber concentrations of mustard gas ranging from 30 to 350 mg/m^3 (5–54 ppm) (Cameron et al. 1946). While incidence data were not provided, Cameron et al. (1946) reported that mustard gas vapor produced lethal effects in rabbits and monkeys in the absence of lung damage, indicating that lethal doses may be absorbed through the mucous membrane of the nose.

In animals, no deaths attributable to mustard gas were noted in mice, rats, guinea pigs, rabbits, or dogs exposed for up to 1 year to 0.1 mg/m^3 (0.015 ppm) (McNamara et al. 1975). Complete experimental details were not provided in this report from the Army.

3.2.1.2 Systemic Effects

The highest NOAEL and all LOAEL values for each study for systemic effects in each species are recorded in Table 3-1 and plotted in Figure 3-1.

Respiratory Effects. Respiratory effects have been found in humans following acute and chronic exposures. Under warm environmental conditions, the respiratory effects of mustard gas were observed to be increased. While durations and levels are not known, toxicologic analyses indicated that some soldiers were exposed to mustard gas during the Iran-Iraq War (1980–1988) (Momeni et al. 1992). Soldiers reported shortness of breath and early respiratory manifestations including hemorrhagic inflammation of the tracheobronchial mucosa accompanied by severe erosions or membranous lesions. Some exposed soldiers became temporarily aphonic due to an acid-like burning sensation of the vocal cords. Brush smears from the trachea and main bronchi showed irregular ciliary beating with lower mean frequencies (9 Hz on average) compared to the normal 10–20 Hz range. In children, cough was the first respiratory symptom (Momeni and Aminjavaheri 1994). Secondary complications consisted of extensive stenosis of sections or the entire tracheobronchial tree, suppurative bronchitis, and chronic respiratory infections with *Staphylococcus aureus*, *Hemophilus influenzae*, and *Pseudomonas aeruginosa* resistant to appropriate antibiotic therapy. Scars, ulcers, strictures, and nonspecific fibrous granulation developed in central airways after a delay up to 15 months. Progressive deterioration of lung compliance and gas exchange with resulting hypoxemia and hypercapnia, were common with injury. Chronic respiratory complaints included shortness of breath, chest tightness, cough, sneezing, rhinorrhea, and sore throats (Momeni et al. 1992; Somani and Babu 1989). Long-term or delayed effects included central airway

Table 3-1. Levels of Significant Exposure to Mustard Gas - Inhalation

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference Chemical Form
ACUTE EXPOSURE							
Systemic							
1	Mouse (albino)	1 h	Renal		21.3 F increased blood and urine uric acid levels		Kumar and Vijayaraghavan 1998
2	Mouse (albino)	1 h	Resp		84.6 F decreased lung/Bd Wt ratio		Pant and Vijayaraghavan 1999
			Bd Wt		84.6 F 14% reduction		
3	Mouse (albino)	1 h	Resp		21.3[b] F decreased respiratory frequency		Vijayaraghavan 1997
			Bd Wt			16.9 F 28% reduction	
4	Gn Pig Not reported	10 min	Bd Wt		125 14% reduction		Allon et al. 1993
Immunological/Lymphoreticular							
5	Mouse (albino)	1 h			84.6 F decreased spleen/Bd Wt ratio		Pant and Vijayaraghavan 1999

[a]The number corresponds to entries in Figure 3-1.

b Used to derive an acute inhalation MRL of 0.0002 mg/m^3; concentration duration adjusted to a 24-hour exposure period, dosimetrically adjusted for humans, and divided by an uncertainty factor of 300 [10 for use of a LOAEL, 3 for extrapolation from animals to humans using a dosimetric adjustment, and 10 for human variability] and a modifying factor of 3 [for proximity to serious effects (28% body weight loss at 16.9 mg/m^3)] (see Appendix A).

Bd Wt = body weight; F = female; Gn Pig = Guinea Pig; h = hour(s); LOAEL = lowest-observed-adverse-effect level; mg/m3 = milligram per cubic meter; NOAEL = no-observed-adverse-effect level; Resp = respiratory

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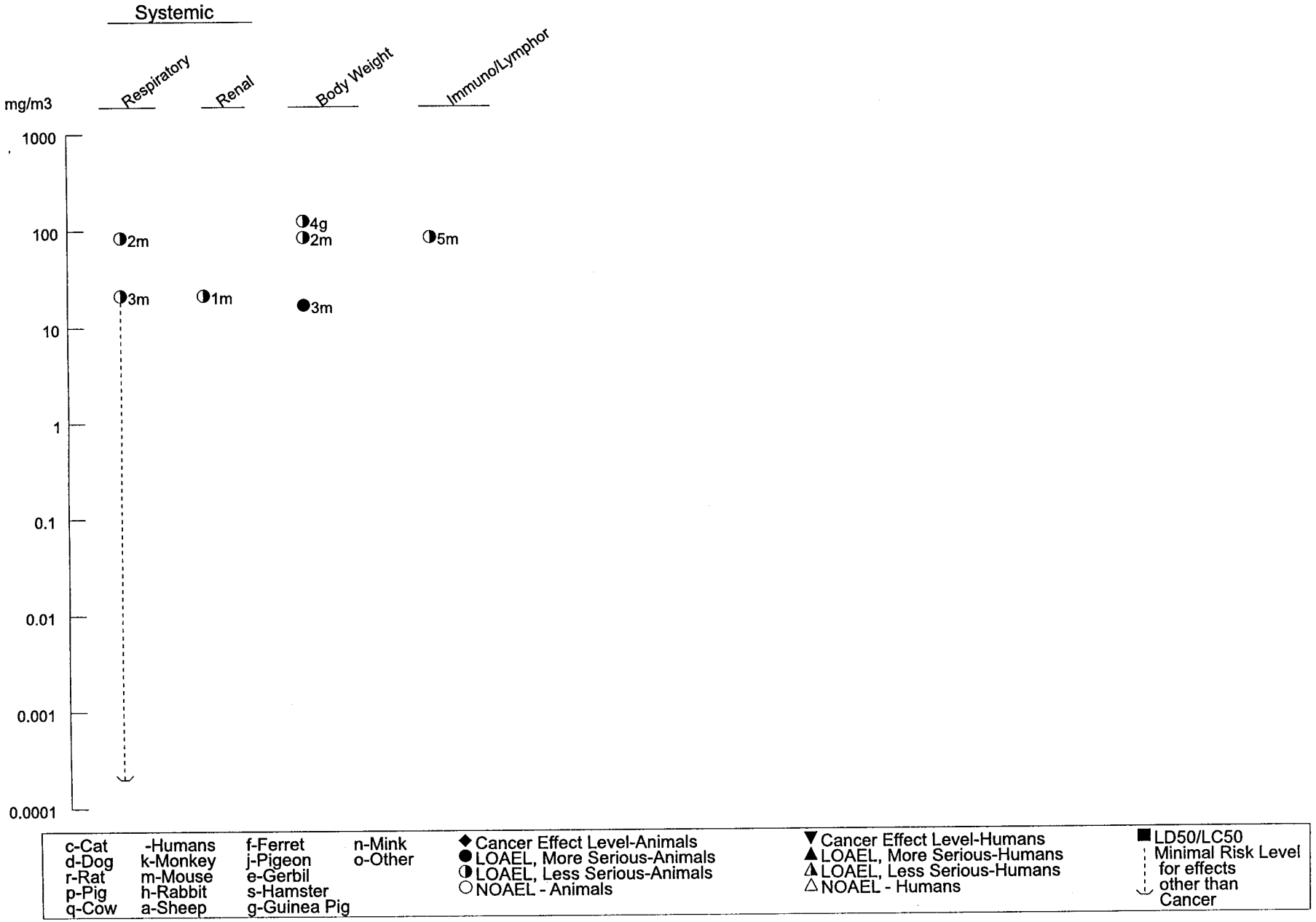
Figure 3-1. Levels of Significant Exposure to Mustard Gas - Inhalation

Acute (≤14 days)



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stenosis, bronchiectasis, bronchiolitis, and bronchitis. British soldiers exposed to mustard gas during combat in World War I had a significantly higher incidence of death due to bronchitis than the general population (Case and Lea 1955).

A group of patients, including a subgroup of 14 children and teenagers (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years), were examined 18–24 hours following exposure to mustard gas from air bombs during the Iran-Iraq War (Momeni and Aminjavaheri 1994). Cough, an early symptom and the most prevalent acute respiratory effect, developed in 11 children (79%). Other respiratory effects included crepitation (57%), dyspenea (57%), wheezing (36%), and sore throat (14%). Children had higher occurrences of respiratory effects than adults.

Other studies show that factory workers who were apparently exposed to mustard gas for a few years (exact quantity and duration not reported) developed acute and chronic respiratory effects. Factory workers in Britain who were exposed to mustard gas also showed increased deaths due to acute and chronic nonmalignant respiratory disease, including influenza and pneumonia (Easton et al. 1988). Workers in a Japanese poison gas factory were more likely to have chronic bronchitis, chronic cough, and decreased respiratory volume than nonexposed persons (Nishimoto et al. 1970). Manning et al. (1981) reported a significantly increased incidence of mortality from pneumonia among 428 former workers of a mustard gas manufacturing facility.

The development of chronic destructive pulmonary sequelae, 10 years after mustard gas vapor exposure, was reported in a clinical study of 197 veterans, admitted to the hospital in 1986 due to acute respiratory symptoms (Emad and Rezaian 1997). The patients' exposures to mustard gas were verified by studies of their urine and vesicular fluid. Patients were included in the study only if they had no history of smoking, cardiovascular disease, asthma, or exposure to other environmental agents known to cause interstitial lung disease or extrinsic allergic alveolitis. A control group was composed of 84 nonsmoking veterans who had participated in the Iran-Iraq War in another region in the same year (1986) but had no exposure to mustard gas. All study subjects had an ECG, a chest x-ray, a high-resolution CT of the chest, pulmonary function testing, and carbon monoxide diffusion capacity testing. Drug treatment was withdrawn for appropriate times prior to testing. Bronchial biopsies were done for each subject. Transbronchial lung biopsy was performed for 24/197 patients whose test data were suggestive of interstitial lung disease. The mean ages of the exposed and control groups were 34.39 and 35.52, respectively. Asthma was newly diagnosed in 21 (10.7%), chronic bronchitis in 116 (58.9%),

bronchiectasis in 17 (8.6%), airway narrowing due to scaring or granulation tissue in 19 (9.6%), and pulmonary fibrosis was observed in 24 (12.2%). None of these were found in the control group, with the exception of a single case of chronic bronchitis. A significant positive correlation was reported between the age of the subject and the severity of asthma, but not with the severity of pulmonary fibrosis. There was a significant correlation between age and incidence, but not the severity, of chronic bronchitis. There was a significant correlation between the severity of pulmonary fibrosis with the spirometry measurement of carbon monoxide diffusion capacity, but not the other physiological parameters of forced vital capacity (FVC) or forced expiratory volume in 1 second (FEV_1). No bronchial carcinoma or other lung malignancies were found in the veterans 10 years after exposure to mustard gas.

A retrospective mortality study was conducted in World War II veterans who participated in U.S. military experiments testing the effectiveness of various protective clothing and equipment in preventing injury due to mustard gas (Bullman and Kang 2000). The study identified 1,545 white male Navy recruits who were exposed to nonlethal levels (>120–960 mg-minute/L) of mustard gas at a single site between 1944 and 1945. A control group consisted of 2,663 white male Navy veterans who served at the same location and time as the exposed, but did not participate in mustard gas chamber tests. Mustard gas chamber test documentation included concentration of mustard gas in the chamber, length of exposure, and subject physiological reactions, so that a dose-response analysis could be conducted. The veterans who participated in the mustard gas chamber tests, while exposed to lower levels than estimated for combat exposed World War I veterans, did have sufficient exposure to produce skin reactions of erythema and edema. Causes of death investigated included laryngeal, lung, and skin cancers, chronic obstructive pulmonary and parenchymal respiratory diseases, external causes, and suicide. While reported as not statistically significant, the greatest mortality rate ratio, 1.57 (95% confidence interval [CI]=0.70–3.54) resulted for chronic obstructive pulmonary disease among veterans with exposure levels in the range of 121–960 mg-minute/L.

Short-term respiratory effects similar to those described in humans have been reported in experimental animals. Microscopic changes of the airways and lungs were reported in rabbits exposed to mustard gas vapor (Pechura and Rall 1993). Correlation of pathology with dose and time after exposure are not possible because of the wide variation in the concentration-time products employed and in the times of examination. However, the major pathological changes were in the nasal passages, pharynx, larynx, and upper portion of the respiratory tract, in some cases, the mouth, and in severe cases, the bronchi and bronchioles. These changes included superficial degeneration or necrosis of the epithelial lining with

pseudomembrane formation accompanied by congestion, edema, and increased mucus secretion. In animals with severe injuries that survived beyond a few days, the lesions incurred secondary infections, leading to bronchopneunomia, which apparently was the cause of death in many cases.

Dogs exposed to unspecified levels of mustard gas developed irregular respiration 8 hours after exposure (Winternitz and Finney 1920). Animals that died 1–3 days after exposure displayed destruction of the epithelial lining, the presence of pseudomembrane, and leukocytic infiltration in the trachea and bronchi. Evidence of bronchopneunomia was present in dogs that died 2–10 days after exposure.

These reports indicate similar respiratory effects of mustard gas in the three species (rabbits, dogs, and human), which suggests that knowledge obtained regarding respiratory effects in animal models can be usefully applied to humans.

Mice exposed to mustard gas vapor exhibited acute and delayed respiratory effects (Vijayaraghavan 1997). Groups of Swiss albino mice (four mice/group, 24–26 g) were administered mustard gas (>99% purity, dissolved in acetone and nebulized) 1 time by inhalation (head only) to 8.5, 16.9, 21.3, 26.8, 42.3, or 84.7 mg/m^3 for 1 hour. At all mustard gas concentrations administered, mice exhibited sensory irritation, 15–20 minutes after the start, characterized by a pause between inspiration and expiration. The respiratory frequency decreased to a slower steady state after 30 minutes of exposure, a decrease of approximately 20% at the lowest concentration to a maximum of 64% for concentrations $42.3 mg/m^3. The concentration that depressed 50% of the respiratory frequency (RD_{50}) was calculated as 27.4 mg/m^3. Normal respiration pattern was recovered after inhalation exposure was terminated; no pauses between respiratory cycles were measured at any exposure level, from which the authors concluded a lack of pulmonary irritation and toxic effects limited to the upper respiratory tract. While sensory irritation was reversible, delayed effects of mustard gas were indicated by a significant reduction in respiratory frequency beginning 48 hours after exposure at concentrations of 21.3 mg/m^3 and higher. The depression in respiratory frequency following exposure was related to both concentration and postexposure time. Airflow limitation was evidenced by a lengthening of expiration time and a decreased respiratory rate and is thought to occur due to the effect of mustard gas on the tracheal secretory cells. Reversible respiratory effects were also observed in similar experiments in mice by Rao et al. (1999) (10.6–42.3 mg/m^3) and by Pant and Vijayaraghavan (1999) (84.6 mg/m^3).

Guinea pigs were exposed by inhalation to 1,200–1,900 µg-minute/L of mustard gas for 10 minutes (120–190 mg/m^3) (Allon et al. 1993). A decrease in respiratory rate and minute volume, and an increase in tidal volume occurred immediately after the onset of exposure and lasted for up to 7 days after exposure. The changes in respiratory parameters were accompanied by a significant reduction in oxygen diffusion capacity in the lung. A dose-related decrease in body weight (14–27%) was also observed, with no recovery evident at 7 days postexposure. Pant and Vijayaraghavan (1999) measured a significant 13% reduction in lung-to-body weight ratio in mice exposed to 84.7 mg/m^3 for 1 hour.

Cardiovascular Effects. In 12 of 53 (23%) autopsies of victims of the World War II Bari Harbor incident during which mustard gas was released in to the air and water, small sub-epithelial hemorrhages were noted in the hearts, but in all instances, the parietal pericardium showed no pathology (Alexander 1947). There was a slight increase in the pericardial fluid having normal color in four cases (8%). In 18 cases, the myocardium was described as pale and lacking normal firmness.

Studies of 65 mustard gas casualties of the Iran-Iraq War treated in European hospitals did not indicate any heart abnormalities (Willems 1989). However, mild tachycardia without fever was reported in a group of 14 children and teenagers (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years) that were examined in a hospital in Iran 18–24 hours following exposure to mustard gas from air bombs during the Iran-Iraq War (incidence not reported) (Momeni and Aminjavaheri 1994). In a 1996 follow-up study of Iran-Iraq War veterans, 10 years after hospital admission in 1986 due to acute respiratory symptoms with confirmed mustard gas exposure, only 3/212 (1.4%) had cardiovascular disease, which was not confirmed attributable to exposure (Emad and Rezaian 1997) (see study description under Respiratory effects).

Gastrointestinal Effects. Victims of the World War II Bari Harbor incident, during which mustard gas was released in to the air and water, suffered local lesions of the oropharynx and upper portion of the esophagus (Alexander 1947). In a few cases, there was intense congestion of the first inch of the esophagus, which may or may not have been due to the blast. In 19 of 53 (36%) cases autopsied, stomach irritation and inflammation were documented. The lesions varied from simple hyperemia, to focal loss of epithelium, necrosis, and ulceration. Some lesions were located near the cardiac end, but most were in the region of the pylorus. In some cases, the hyperemia extended into the duodenum, and in one case, congestion of the jejunum was noted (Alexander 1947). Incidences of gastrointestinal effects of nausea (9 patients, 64%), vomiting (6 patients, 43%), and bleeding (2 patients, 14%) were reported in

a group of 14 children and teenagers (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years) that were admitted to a hospital in Iran 18–24 hours following exposure to mustard gas from air bombs during the Iran-Iraq War (Momeni and Aminjavaheri 1994). In a review of the clinical manifestations of mustard gas exposure in the Iran-Iraq War victims, Pierard et al. (1990) reported that endoscopy frequently revealed acute gastritis. Gastrointestinal neoplasms were reported in Japanese mustard gas factory workers who were involved with the production of chemical agents during World War II (Yamakido et al. 1985). Angelov et al. (1996) observed changes in the intestinal muscosa consisting of villi necrosis, dilatation of blood vessels, and increased cellular presence in broiler chickens after inhalation exposure to 0.9 mg/L (900 mg/m^3, 138 ppm) of mustard gas for 30 minutes.

Hematological Effects. There are reports of changes in white blood cell (WBC) counts in victims of mustard gas exposure during World War I and the Iran-Iraq War. During days 1–3 following exposure in World War I, increases of 3–5 times normal levels in WBC counts in peripheral blood were measured (Marrs et al. 1996). The increase was due mainly to an increase in polymorphonuclear cells, while lymphocytes were reduced in numbers during this period. In severe cases, a subsequent leukopenia occurred with WBC counts falling to <200 cells/µL. Leukopenia was also observed in casualties of mustard gas exposure of the World War II Bari harbor incident and during the Iran-Iraq War (Dacre and Goldman 1996; Marrs et al. 1996; Momeni and Aminjavaheri 1994). A 13-month-old Iranian girl developed pancytopenia and respiratory failure, and died 8 days after exposure (Momeni and Aminjavaheri 1994). In a group of 14 children and teenagers (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years) that were admitted to a hospital in Iran 18–24 hours following exposure to mustard gas from air bombs during the Iran-Iraq War, admission WBC counts ranged from 9,500 to 11,200 cells/µL, indicating mild leukocytosis (Momeni and Aminjavaheri 1994).

In a review of the clinical manifestations of mustard gas exposure in the Iran-Iraq War victims, Pierard et al. (1990) reported that in addition to the leukocytosis followed by leukopenia and lymphopenia described above, the ratio of T and B lymphocytes decreases, while the phagocytic function of neutrophils remains intact. A primary decrease in albumin and increase in α-globulin content, especially α_1 antitrypsin, occurs. Both C3 and C4 titers first increase, followed by a gradual decrease. Aplastic anemia is not uncommon. Increases in serum tumor markers, α-fetoprotein, β-HCG, and CA-125, have been observed, but the relevance of these increases to the oncogenic potential of mustard gas is not yet known.

The spleen demonstrated evidences of pathology in 33 of 53 (62%) autopsies of victims of the World War II Bari Harbor incident during which mustard gas was released in to the air and water (Alexander 1947). The majority were described as shrunken in size with pale color. Discoloration of the lymph nodes in the axillary, inguinal, and mesenteric glands were noted. The bone marrow was only examined in one autopsy and a pale pink color was described.

Cameron et al. (1946) provided a general description of pathological changes in rabbits and monkeys that had undergone tracheal cannulation and were exposed to nominal chamber concentrations of mustard gas ranging from 30 to 350 mg/m^3 (5–54 ppm). A large blood clot occupied most of the nasal passages in severe cases. In milder cases, damage was found only in the anterior turbinate bones and consisted of small hemorrhages in congested mucous membrane, with polymorphonuclear infiltration. The glands of Bowman and the organ of Jacobsen were consistently uninjured. Mustard gas vapors most often had little effect on the lungs; however, lethal effects occurred in the absence of lung damage.

Dogs and rabbits exposed to 0.1 mg/m^3 of mustard gas in the air for 1 year showed no hematological changes in a study that did not report further experimental details (McNamara et al. 1975).

Changes in the coloring and formation of erythrocyte nuclei and fatty dystrophy of bone marrow cells were observed in broiler chickens after inhalation exposure to 0.9 mg/L (900 mg/m^3, 138 ppm) of mustard gas for 30 minutes (Angelov et al. 1996).

Blood uric acid increased significantly in a dose- and time-related manner in female mice exposed by inhalation to 21.2, 42.3, or 84.6 mg/m^3 of mustard gas for 1 hour (Kumar and Vijayaraghavan 1998). Blood uric acid levels peaked at 2 days after exposure, but were still significantly elevated above controls at 7 days postexposure.

Musculoskeletal Effects. No evidence of mustard gas-related changes to the musculoskeletal system was reported in any of 53 autopsies of victims of the World War II Bari Harbor incident during which mustard gas was released in to the air and water (Alexander 1947).

Hepatic Effects. In 39 of 53 (74%) autopsies of victims of the World War II Bari Harbor incident, during which mustard gas was released in to the air and water, yellow streaks and patches grossly appearing as patchy fatty necrosis were observed throughout the liver (Alexander 1947). Several pale

liver sections and atypical liver texture were mentioned. In 3 of 53 (6%) autopsies, small subcapular hemorrhages, and in one instance, a small rupture near the diaphragmatic attachment, were noted. The gall bladder contained thick inspissated bile. Microscopic examinations were performed on 31 of the 39 livers with gross changes. Five showed fatty change and two showed focal necroses.

Renal Effects. Renal complications, consisting of acute hemorrhagic nephritis, oliguria, albuminuria, and casts, have been reported in near-death stages of mustard gas warfare victims (Papirmeister et al. 1991).

In 25 of 32 (78%) kidneys from Bari Harbor incident casualties, microscopic examinations revealed tubular casts, 3 were calcified, 8 showed hemoglobin casts, and in 14, both types were present (Alexander 1947).

Blood uric acid increased significantly in a dose- and time-related manner mice, indicative of kidney damage, in females exposed by inhalation to 21.2, 42.3, or 84.6 mg/m^3 of mustard gas for 1 hour (Kumar and Vijayaraghavan 1998). Blood uric acid levels peaked at 2 days after exposure, but were still significantly elevated above controls at 7 days postexposure.

Endocrine Effects. No significant findings were noted grossly in the thyroid or adrenal glands in any of 53 autopsies of victims of the World War II Bari Harbor incident (Alexander 1947).

The time course of changes in serum concentrations of total and free testosterone, lutenizing hormone (LH), dehydroepiandrosterone (DS), follicle-stimulating hormone (FSH), 17 α-OH progesterone, and prolactin were studied in 16 men during the first 3 months after chemically confirmed exposure in 1987 during the Iran-Iraq War to chemical weapons containing mustard gas (Azizi et al. 1995). A group of 34 healthy unexposed men of similar age served as controls. At 1 week after exposure, total testosterone, free testosterone, and DS were significantly lower, 57, 72, and 53%, respectively, in exposed men than in controls, while levels of the remaining hormones were comparable between groups. Total testosterone, free testosterone, and DS levels continued to decrease during the first 5 weeks after exposure. At 1 week, 4 of 16 exposed men (25%) had serum testosterone levels that were reduced by >60% below the control average; by the 5^{th} week, the number increased to 11 (69%). DS mean values reached as low as 18% of the mean of control subjects. After the 5^{th} week, these three hormone levels increased returning to normal levels at 12 weeks after injury. Small but significant increases in mean

serum concentration of LH at the 3rd week and that of FSH and prolactin at the 5th week, were measured. Normal levels of LH, FSH, and prolactin were measured at 12 weeks. FSH and LH response levels to 100 µg of gonadotropin releasing hormone (GnRH) administered intravenously during the first week after exposure, were subnormal in four of five patients.

In another study, the time course of changes in thyroid indices, serum T3, T4, TSH, reverse T3, thyroglobulin and cortisol, plasma adrenocorticotropic hormone (ACTH), and free T3 and T4 indexes (FT3I, FT4I) were studied in 13 male soldiers, ages 21–32 years, during the first 5 weeks after chemically confirmed exposure in 1987 during the Iran-Iraq War to chemical weapons containing mustard gas (Azizi et al. 1993). A group of 34 healthy unexposed men of similar age served as controls. T4 and FT4I were not consistently affected following injury; compared to controls, significantly decreased values were measured at 1 and 5 weeks after exposure, but values slightly above normal were measured at 3 weeks. T3 and FT3I were significantly lower (11–23%) than control at 1, 3, and 5 weeks after injury. Reverse T3 concentration in injured men was significantly higher (29%) than mean control value at 1 week, but was normal at weeks 3 and 5. TSH and thyroglobulin levels in the injured soldiers were comparable to controls during the 5 postexposure weeks. Cortisol was significantly higher (40%) than normal 1 week after exposure, within the normal range at week 3, and significantly decreased (50%) below normal at week 5. ACTH was significantly increased (57–80%) above the normal control value at 1, 3, and 5 weeks after exposure.

In a follow-up study of 42 men, ages 18–37, injured by mustard gas during the Iran-Iraq War, serum testosterone, LH, and prolactin concentrations were normal in all men 1–3 years following exposure (Azizi et al. 1995). A comparison of the mean serum FSH concentration in 13 subjects with sperm count below 20 million and in 20 subjects with sperm counts above 60 million, revealed a nearly 2-fold increase in FSH concentration in the those with the lower sperm count; the increased FSH level was 38% above the mean FSH concentration in a group of 34 health unexposed males.

Dermal Effects. The U.S. Army Soldier and Biological Chemical Command (SBCCOM 1999) reports a maximum safe concentration time product (Ct) of 5 mg-minute/m^3 for human skin exposure to mustard gas. The ICt_{50} (estimated concentration-exposure time period product incapacitating to 50% of exposed individuals) for human skin exposure is dependent on temperature, 2,000 mg-minute/m^3 at 70–80 EF (humid environment) and 1,000 mg-minute/m^3 at 90 EF (dry environment) (SBCCOM 1999).

Ocular Effects. In humans, an ICt_{50} and a maximum safe Ct for eye exposure are 200 and 2 mg-minute/m^3, respectively, for mustard gas (SBCCOM 1999).

Body Weight Effects. In female Swiss albino mice exposed to mustard gas for by inhalation (head only) at concentrations of 8.5, 16.9, 21.3, 26.8, 42.3, or 84.7 mg/m^3 for 1 hour, decreases in body weight began 24 hours after exposure, were concentration-related, and achieved statistical significance ($p<0.05$) at concentrations of 16.9 mg/m^3 or higher (Vijayaraghavan 1997). At 7 days postexposure, body weights were decreased by 2, 13, 28, 25, 32, and 34% in the control, 8.5, 16.9, 21.3, 26.8, and 42.3 mg/m^3 exposure groups. In another study in female albino mice, in which mustard gas was administered at 84.6 mg/m3 for 1 h., a progressive fall in body weight was observed starting on the third post-exposure day, and at post-exposure day 7, body weight was significantly reduced by 14%, compared to control animals (Pant and Vijayaraghavan 1999). Food and water intake was also significantly decreased.

Guinea pigs administered nominal concentrations of 1250, 1650, or 1750 µg-min/l (125, 165, or 175 mg/m^3) of mustard gas (head only) for 10 minutes exhibited a dose-related significant decrease in body weight, with no recovery evident at 6-7 days post-exposure (Allon et al. 1993). At 6-7 days post-exposure, body weight was reduced compared to controls by -14%, -24%, and -27% at the low-, mid-, and high-concentrations, respectively (data presented graphically).

3.2.1.3 Immunological and Lymphoreticular Effects

The spleen demonstrated evidences of gross pathology in 33 of 53 (62%) autopsies of victims of the World War II Bari Harbor incident during which mustard gas was released in to the air and water (Alexander 1947). In the majority of cases, the spleen was described as shrunken in size with pale color. Discoloration of the lymph nodes in the axillary, inguinal, and mesenteric glands were noted. No significant findings were noted grossly in the thymus in any of the autopsies. Microscopically only 2 of 32 spleens examined showed degeneration or necrosis; pyknosis and karyorrhexis of lymphocytes in some corpuscles was observed in one and slight necrosis of the malpighian follicle in the other.

Cameron et al. (1946) provided a general description of pathological changes in rabbits and monkeys that had undergone tracheal cannulation and were exposed to nominal chamber concentrations of mustard gas ranging from 30 to 350 mg/m^3 (5–54 ppm). After 12 hours, damage was found in the cervical lymph nodes, which drain the nose and lymphoid tissue throughout the body. In experiments where the time

sequence was studied, damage to the cervical lymph nodes could not be attributed solely to lymphatic absorption from nasal mucosa, since identical changes resulted from topical skin application or subcutaneous injection of mustard gas.

Angelov et al. (1996) detected atrophy of the lymphoid tissue in the bursa Fabricii of broiler chickens after inhalation exposure to 0.9 mg/L (900 mg/m^3, 138 ppm) of mustard gas for 30 minutes.

Pant and Vijayaraghavan (1999) measured a significant 38% reduction in spleen-to-body weight ratio in mice exposed to 84.7 mg/m^3 for 1 hour.

3.2.1.4 Neurological Effects

Nausea and vomiting are acute responses to mustard gas exposure in adults and children. Nausea and vomiting occurred, respectively, in 9 (64%) and 6 (43%) of 14 Iranian children admitted to a hospital following exposure to mustard gas from air bombs during the Iran-Iraq War (Momeni and Aminjavaheri 1994).

No significant findings were noted grossly in the central nervous system in any of 53 autopsies of victims of the World War II Bari Harbor (Alexander 1947).

Dogs exposed to unspecified levels of mustard gas showed no tremors or convulsions (Winternitz and Finney 1920).

3.2.1.5 Reproductive Effects

In a follow-up study of 42 men, ages 18–37, conducted 1–3 years after injury by mustard gas during the Iran-Iraq War, the mean sperm count was 84 million cells per mL, ranging from 0 to 328 million cells per mL (Azizi et al. 1995). Thirteen (29%) had decreased sperm count below 20 million. Serum testosterone, LH, and prolactin concentrations in the 13 subjects with sperm count below 20 million were comparable to the levels in 20 subjects with sperm count above 60 million. FSH measured in these same groups was higher in the group with lower sperm counts. The increased FSH level was 38% above the mean FSH concentration in a group of 34 healthy unexposed males. Complete or relative arrest of

spermatogenesis was evident in each testicular biopsy (100% incidence) performed on six men with sperm count below 20 million cells per mL.

Pour-Jafari (1992, 1994a) reported an increased rate of fetal deaths and an increased secondary sex ratio (57.2 vs. 51.0% in controls, percent of males) in progenies of Iranian survivors of chemical attacks that included mustard gas.

In a survey of 800 Iranian men who were exposed to mustard gas during the Iran-Iraq War, 279 men (34.8%) reported decreased libido, 342 (42.8%) reported no change, 6 (0.8%) reported increased libido, and 173 (21.6%) did not respond to this survey question (Pour-Jafari and Moushtaghi 1992). Of these men, 86.6% still suffered symptoms from chemical injury, namely lung and skin lesions.

Chronic (52 weeks) inhalation exposure of male rats to mustard gas (0.1 mg/m^3) was reported to produce significant dominant lethal mutation rates (a maximum of 9.4% at 12–52 weeks), but exposure of pregnant females to the same concentration for a shorter time interval did not (Rozmiarek et al. 1973). McNamara et al. (1975) subsequently concluded from these same data that there were no differences between the control and experimental groups and no evidence of mutagenesis. The conflict between these two reports is not readily resolvable, but the fetal mortality values presented by McNamara et al. (1975) suggest at least a trend for dominant lethal effect. Complete control data and statistical analyses of the results were not presented, but percentages of fetal death at week 12 were 4.12, 4.24, and 21.05 for controls, 0.001, and 0.1 mg/m^3 exposure groups, respectively.

3.2.1.6 Developmental Effects

Pour-Jafari (1994b) reported an increased incidence of congenital malformations among offspring of Iranian mustard gas victims.

No excess in fetal abnormalities were noted when rat dams were exposed to mustard gas by inhalation during gestation (McNamara et al. 1975). This study had a number of drawbacks, including failure to report humidity in the chamber (which made an adequate assessment of exposure levels difficult), whole-body exposure of animals (which made the exposure a combination of inhalation as well as ingestion effects), additions of animals in the chamber midway through the experiment (which allowed for entrance of pathological organisms), lack of rationale for the selection of dosages, lack of details of

pathological examination (which normally includes description of the types of defects the investigators were looking for), lack of information about historical controls, and lack of statistical evaluations. In particular, the authors stated that the fetuses were examined, but they did not indicate whether or not there were any fetal abnormalities.

3.2.1.7 Cancer

Human Cancer Studies. Data on cancer in humans after inhalation exposure to mustard gas are from two primary sources: inhalation for several years by mustard gas factory workers and inhalation as the result of a few or of single exposures during combat in World War I and in the Iran-Iraq War. While several epidemiologic studies provide sufficient evidence that mustard gas is carcinogenic in humans, particularly in the upper respiratory tract, in no case was the exposure level or duration quantified, and therefore, these data are inadequate for deriving dose-response relationships. Typically, factories produced several different poisonous gases and workers involved with mustard gas production were exposed to other toxic chemicals, confounding any study findings.

Other studies provide epidemiological evidence that World War I veterans who were exposed to mustard gas in combat had slight, but statistically significant, increased incidences of lung cancer deaths. British retired veterans who were studied 15 years after their exposure to mustard gas in World War I showed twice the expected number of deaths due to lung cancer (standard mortality ratio [SMR]=2; $p<0.01$) compared to controls and also had excessive deaths from bronchitis (SMR=10, $p<0.001$), as compared to nonexposed soldiers (Case and Lea 1955). The authors suggest that the increased lung cancer was due to the bronchitis and not directly to the mustard gas. Veterans who were not exposed to mustard gas, but who did have bronchitis also had excess mortality due to lung cancer (SMR=2; $p<0.01$), as compared with controls. Deaths from neoplasms other than cancer of the lung were not significantly increased.

A cohort of American World War I soldiers was studied 1–37 years (Beebe 1960) and 47 years (Norman 1975) postexposure. Deaths from respiratory cancer occurred in 2.5% of those exposed to mustard gas, 1.8% of those having pneumonia, and in 1.9% of a control group (Norman 1975). The respiratory cancer rate ratio of 1.3 (95% CI=0.9–1.9) is suggestive evidence of an association of lung cancer with mustard gas exposure. These studies found no association of lung cancer with bronchitis, in contrast to the findings of Case and Lea (1955). Although an increased frequency of lung cancer is associated with

exposure to mustard gas, it is very difficult to calculate attributable risk of lung cancer due to mustard gas, as no control was made for cigarette smoking.

A retrospective mortality study was conducted in World War II veterans who participated in U.S. military experiments testing the effectiveness of various protective clothing and equipment in preventing injury due to mustard gas (Bullman and Kang 2000). The study identified 1,545 white male Navy recruits who were exposed to nonlethal levels (>120–960 mg-minute/L) of mustard gas at a single site between 1944 and 1945. A control group consisted of 2,663 white male Navy veterans who served at the same location and time as the exposed, but did not participate in mustard gas chamber tests. Mustard gas chamber test documentation included concentration of mustard gas in the chamber, length of exposure, and subject physiological reactions, so that a dose-response analysis could be conducted. The veterans who participated in the mustard gas chamber tests, while exposed to lower levels than estimated for combat exposed World War I veterans, did have sufficient exposure to produce skin reactions of erythema and edema. Causes of death investigated included laryngeal, lung, and skin cancers, chronic obstructive pulmonary and parenchymal respiratory diseases, external causes, and suicide. The mortality rate ratios for all cancer types among the total exposure group and all subgroups were less than unity. The greatest mortality rate ratio, 1.57 (95% CI=0.70–3.54) resulted for chronic obstructive pulmonary disease among veterans with exposure levels in the range of 121–960 mg-minute/L. The authors indicated that this value was not statistically significant and that there was no excess of any cause-specific mortality associated with mustard gas exposure or associated with level of mustard gas exposure among veterans. The authors noted that reliance on death certificates for cause of death and lack of data on potential confounders (smoking, drinking habits, and occupational history/exposure to carcinogens) were potential study weaknesses.

In a 1996 follow-up clinical study of 197 Iran-Iraq War veterans, 10 years after hospital admission in 1986 due to acute respiratory symptoms with confirmed mustard gas exposure, no bronchial carcinoma or other lung malignancies were found (Emad and Rezaian 1997) (see study description under respiratory effects in Section 3.2.1.2).

Studies from three countries show elevated incidence of lung cancer among factory workers who made mustard gas and other chemical agents. In Japanese factory workers, histological examination revealed foci of moderate or severe atypical cell lesions or carcinoma in the bronchial epithelium (Tokuoka et al. 1986). Another study of workers from this same factory showed an increased number of deaths

(SMR=37; 33 deaths observed vs. 0.9 deaths expected) from cancer of the respiratory passages (Wada et al. 1968). The neoplasms were of either the squamous or undifferentiated type. In another study of Japanese factory workers, with estimated factory mustard gas concentrations of 0.05–0.07 mg/L (0.0017–0.0024 mg/kg/day, for an average 70 kg body weight, 8-hour day, and 5-day work week) (Nakamura 1956), of 172 worker deaths, 48 (28%) were due to malignant tumors compared with 7.7 and 8.5% in two groups of unexposed residents of the same area (Yamada 1963). Respiratory tract tumors accounted for 58% of all malignant tumors (16% of all deaths). In the two reference groups, the incidence of respiratory tumors was much lower, 0.5 and 0.3%, respectively. In Japanese mustard gas cases, central lung cancers were more commonly observed than peripheral lung cancers, and the most common histologic types were squamous cell carcinoma and small cell carcinoma (Yamada 1963). The duration of mustard gas exposure in cases of lung cancer was 7–9 years, and the latent period for tumor induction was 17–20 years.

Two historical cohort studies were conducted to determine the comparative risk for development of cancer in Japanese males who worked in a poison gas factory between 1927 and 1945 (Nishimoto et al. 1983; Yamakido et al. 1996). The gases produced at the factory included mustard gas, lewisite, diphenylcyanarsine, hydrocyanic acid, chloracetophenone, and phosgene. No estimates of chemical exposures levels were given. However, the workers were divided into three groups according to type of work and association with mustard gas in an attempt to establish a dose-relationship. One group consisted of workers engaged directly in the production of mustard gas and lewisite. A second group consisted of workers who had come into contact with mustard gas and/or lewisite in laboratories or during repair or inspection in the factory. A third group consisted of those engaged directly in the production of the remaining gases, other than mustard gas and lewisite, or who were working in medical or administrative work. Nishimoto et al. (1983) investigated 2,068 cases and found that the number of deaths from cancer of the lungs in the two groups with the highest mustard gas exposure potential was more than 3 times the number in the local male population (SMR$3, $p<0.01$). Deaths due to cancers of the gastrointestinal tract and liver or other type were not significantly elevated. Yamakido et al. (1996) studied 1,632 male workers from this same factory. In this study, in addition to grouping according to type of work, groups were further subdivided according to the duration of work in the factory, <0.5 years, 0.5–5 years, or >5 years. The SMRs for lung cancer were significant ($p<0.001$) in the group working directly in the production of mustard gas and lewisite for >6 months (SMR=3.24 [0.5–5 years], SMR=7.35 [>5 years]). In the second grouping of workers who had less contact with mustard gas, the SMR for lung cancer was significant only in the subgroup with >5 years of employment (SMR=4.92),

further supporting a dose-relationship for lung cancer. However, there were no data presented to relatively weight the exposure to mustard gas and lewisite within the work type grouping; therefore, co-exposure to lewisite confounds the interpretation of these findings. The SMR for all malignant neoplasms was significant in the group with the highest mustard gas exposure potential with >6 months of employment (SMR=1.44 [0.5–5 year, $p<0.05$], SMR=2.36 [>5 year, $p<0.001$]) and in the second grouping of workers who had less contact with mustard gas but worked at the factory for the longest duration (SMR=1.66 [>5 year, $p<0.05$]).

British mustard gas workers also showed increased deaths from cancers of the respiratory passages and from lung cancer (Manning et al. 1981). In a cohort study of 502 workers involved in mustard gas manufacturing between 1940 and 1945, a significant excess mortality was observed for carcinoma of the larynx and trachea (SMR=7.5, $p<0.02$). While not listed as cause of death, seven subjects developed cancer of the larynx, compared with 0.75 expected, yielding a rate ratio of 9.3 ($p<0.001$). Increased mortality due to cancers of other organs was not statistically significant. In another study of 3,354 British mustard gas workers, significant excesses were observed compared with national death rates for deaths from cancer of the larynx (SMR=2.7, 11 deaths observed, 4.04 deaths expected, $p=0.003$), pharynx (SMR=5.5, 15 observed, 2.73 expected, $p<0.001$), lung (SMR=1.4, 200 observed, 138.39 expected, $p<0.001$), upper respiratory sites combined (lip, tongue, salivary gland, mouth, and nose) (SMR=2.8, 12 observed, 4.29 expected, $p=0.002$), esophagus (SMR=1.9, 20 observed, 10.72 expected, $p<0.01$), and stomach (SMR=1.4, 70 observed, 49.57 expected, $p<0.001$) (Easton et al. 1988). The risks of cancers of the pharynx and lung, but not of the esophagus and stomach, were significantly related to duration of employment.

A study of workers in U.S. mustard gas factories reported incidences of chronic bronchitis after 3–6 months of employment, but did not mention any incidence of lung cancer (Morgenstern et al. 1947). German factory workers also showed increases in bronchial carcinoma, bladder carcinoma, and leukemia (Weiss and Weiss 1975).

Animal Cancer Studies. Two animal studies showed tumors following inhalation exposure to mustard gas. Male and female Strain A mice exposed once for 15 minutes to an unquantified level of mustard gas had a significantly higher incidence of pulmonary tumors than did their littermate controls (Heston 1953b). The significance of this finding for humans is difficult to determine since these Strain A mice are used due to their specific genetic tendency to develop lung tumors. Guinea pigs, mice, rabbits, and

dogs that were exposed to mustard gas in the air for 3–12 months did not develop tumors, although rats did develop squamous cell carcinoma of the skin (McNamara et al. 1975). This study used insufficient animals and inadequate doses to be considered an adequate carcinogenesis assay.

3.2.2 Oral Exposure

No studies were located regarding the health effects in humans after oral exposure to mustard gas. While exposure to mustard gas by the oral route can occur, dermal or inhalation exposure is more prevalent.

3.2.2.1 Death

In humans, the LD_{50} for oral exposure is estimated to be 0.7 mg/kg (SBCCOM 1999).

Significant maternal mortality occurred in a teratology study in which mustard gas was administered acutely by oral gavage to mated female animals of two species, rats and rabbits, on gestation days 6 through 15 and 6 through 19, respectively (DOA 1987b). Rabbits were dosed with 0, 0.5, 1.0, 2.0, or 2.5 mg/kg/day of mustard gas in a range-finding study and with 0, 0.4, 0.6, or 0.8 mg/kg/day in the teratology study. In rabbits, maternal mortality was dose-related with mustard gas-related deaths occurring with a dose of 0.8 mg/kg/day or higher, 3/18 (17%) at 0.8 mg/kg/day, 3/7 (43%) at 1.0 mg/kg/day, 5/8 (63%) at 2.0 mg/kg/day, and 4/6 (75%) at 2.5 mg/kg/day. In the range-finding study, female rats were dosed with 0, 0.2, 0.4, 0.8, 1.6, 2.0, or 2.5 mg/kg/day and with 0, 0.5, 1.0, or 2.0 mg/kg/day in the teratology study. One of three rats died on gestation day 12 at the highest dose of 2.5 mg/kg/day. No maternal deaths in rats were attributed to mustard gas at doses below 2.5 mg/kg/day.

No mustard gas-related mortality occurred at doses of 0.5 mg/kg/day or less in three intermediate-duration toxicity studies in rats (Sasser et al. 1993, 1996a, 1996b). In a sub-chronic toxicity study, groups of 6–7-week-old Sprague-Dawley rats (12/sex/group) were orally gavaged with 0, 0.003, 0.01, 0.03, 0.1, or 0.3 mg/kg/day mustard gas in sesame oil, 5 days/week for 13 weeks (Sasser et al. 1996b). In a two-generation study, groups of 8-week old Sprague-Dawley rats (27 female and 20 males/ group/generation) were orally gavaged with 0, 0.03, 0.1, or 0.4 mg/kg/day mustard gas in sesame oil (Sasser et al. 1996a). Male and female rats were dosed 5 days/week for 13 weeks before mating and during a 2-week mating period. Females were dosed daily (7 days/week) throughout the 21-day gestation and parturition period and 4–5 days/week during the 21-day lactation period. Male and female F1 pups

were treated with mustard gas until they were mated and the females became pregnant and gave birth. The dosing of F1 dams continued until pup weaning, at which time, the study was terminated. In a dominant lethal study, groups of 7–10-week-old Sprague-Dawley rats were orally gavaged with 0.08, 0.2, or 0.5 mg/kg/day mustard gas in sesame oil for 5 days/week for 10 weeks (Sasser et al. 1993). In the female dominant lethal phase, groups of rats (40 females and 10 males/group) were administered 0 (sesame oil), 0.08, 0.2, or 0.5 mg/kg/day mustard gas for 5 days/week for 10 weeks prior to a 19-day breeding interval. In the male dominant lethal phase, groups of rats were administered 0 (sesame oil), 0.08, 0.2, or 0.5 mg/kg/day mustard gas for 5 days/week for 10 weeks.

The highest NOAEL and all LOAEL values for each study for death in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

The highest NOAEL and all LOAEL values for each study for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2. No studies were located regarding musculoskeletal, hepatic, renal, or metabolic effects in humans or animals after oral exposure to mustard gas.

Respiratory Effects. Gross examinations of the lungs of rats orally gavaged with 0.3 mg/kg/day mustard gas 5 days/week for 13 weeks did not reveal any significant treatment related lesions (Sasser et al. 1996b).

Cardiovascular Effects. Microscopic examinations of the heart of rats orally gavaged with 0.3 mg/kg/day mustard gas 5 days/week for 13 weeks did not reveal any significant treatment related lesions (Sasser et al. 1996b).

Gastrointestinal Effects. Dose-related gastrointestinal effects have occurred in experimental animals following acute and subchronic oral administration of mustard gas. In mated female rats orally gavaged with 0.2–2.5 mg/kg/day of mustard gas on gestation days 6 through 15, gastric mucosa inflammation was observed in 2/30 (6.7%) and 2/3 (66.7%) rats at 2.0 and 2.5 mg/kg/day, respectively, but not in any of the lower dose or control groups (DOA 1987b). Inseminated female rabbits orally gavaged with 0.4–2.5 mg/kg/day of mustard gas on gestation days 6 through 19 incurred dose-related

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Table 3-2. Levels of Significant Exposure to Mustard Gas - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	ACUTE EXPOSURE						
	Systemic						
1	Rat (Sprague-Dawley)	10 d Gd 6-15 (GO)	Hemato	0.5 F		1.0 F (decrease in hematocrit)	DOA 1987b
			Dermal	2.0 F			
2	Rabbit (NS)	14 d Gd 6-19 (GO)	Hemato	0.6 F		0.8 (decrease in hematocrit)	DOA 1987b
			Dermal	0.8 F			
			Bd Wt	0.6 F	0.8 (7.9-10.5% decrease after 5 days of exposure)		
	Immunological/Lymphoreticular						
3	Rat (Sprague-Dawley)	10 d, Gd 6-15 (GO)			0.5[b] F (inflamed mesenteris lymph nodes)		DOA 1987b
	Developmental						
4	Rat (Sprague-Dawley)	10 d Gd 6-15 (GO)			0.5[b] (reduced ossification)		DOA 1987b
5	Rabbit (NS)	14 d Gd 6-19 (GO)		0.8			DOA 1987b

Table 3-2. Levels of Significant Exposure to Mustard Gas - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
INTERMEDIATE EXPOSURE							
Systemic							
6	Rat (Sprague-Dawley)	10 wk 5 d/wk 1 x/d	Dermal	0.5			Sasser et al. 1993
		(GO)	Bd Wt	0.2	0.5 M		
7	Rat (Sprague-Dawley)	18-21 wk 5 d/wk	Gastro		0.03 (29/47 M, 42/47 F; epithelial acanthosis of the forestomach)		Sasser et al. 1996a
		(GO)	Dermal	0.4			
8	Rat (Sprague-Dawley)	13 wk 5 d/wk 1 x/d	Resp	0.3			Sasser et al. 1996b
		(GO)	Cardio	0.3			
			Gastro	0.03	0.3[c] (5/12 M, 5/12 F; epithelial hyperplasia of the forestomach)		
			Hemato	0.1	0.3 F (8 % decrease in serum protein concentration)		
			Hepatic	0.3			
			Renal	0.3			
			Endocr	0.3			
			Dermal	0.3			
			Bd Wt	0.1	0.3 (>10% decrease in females, >8% decrease in males)		

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Table 3-2. Levels of Significant Exposure to Mustard Gas - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL				Reference Chemical Form
					Less Serious (mg/kg/day)		Serious (mg/kg/day)		
	Immunological/Lymphoreticular								
9	Rat (Sprague-Dawley)	13 wk 5 d/wk 1 x/d (GO)		0.3					Sasser et al. 1996b
	Neurological								
10	Rat (Sprague-Dawley)	10 wk 5 d/wk 1 x/d (GO)		0.2	0.5	(excessive drooling)			Sasser et al. 1993
	Reproductive								
11	Rat (Sprague-Dawley)	10 wk 5 d/wk 1 x/d (GO)					0.5	(2-fold increase in abnormal sperm head morphology)	Sasser et al. 1993
							0.08	(4-fold increase in resorptions; increased preimplantation losses; 7% decrease in live fetuses)	
12	Rat (Sprague-Dawley)	18-21 wk 5 d/wk (GO)		0.1	0.4	(increased fraction of males, 58%)			Sasser et al. 1996a

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Table 3-2. Levels of Significant Exposure to Mustard Gas - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
13	Rat (Sprague-Dawley)	13 wk 5 d/wk 1 x/d (GO)		0.3			Sasser et al. 1996b
	Developmental						
14	Rat (Sprague-Dawley)	18-21 wk 5 d/wk (GO)		0.4			Sasser et al. 1996a

[a]The number corresponds to entries in Figure 3-2.
[b]Used to derive an acute oral minimum risk level (MRL) of 0.0005 mg/kg/day; dose divided by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).
[c]Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.00002 mg/kg-day; by adjusting for intermittent exposure (see Apendix A) and dividing by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = female; gastro = gastrointestinal; Gd = gestation day; (GO) = gavage in oil; Hemato = hematological; LOAEL = lowest-observed-adverse-effect level; M = male; mg/kg/day = milligram per kilogram per day; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); x = times

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Figure 3-2. Levels of Significant Exposure to Mustard Gas - Oral

Acute (≤14 days)

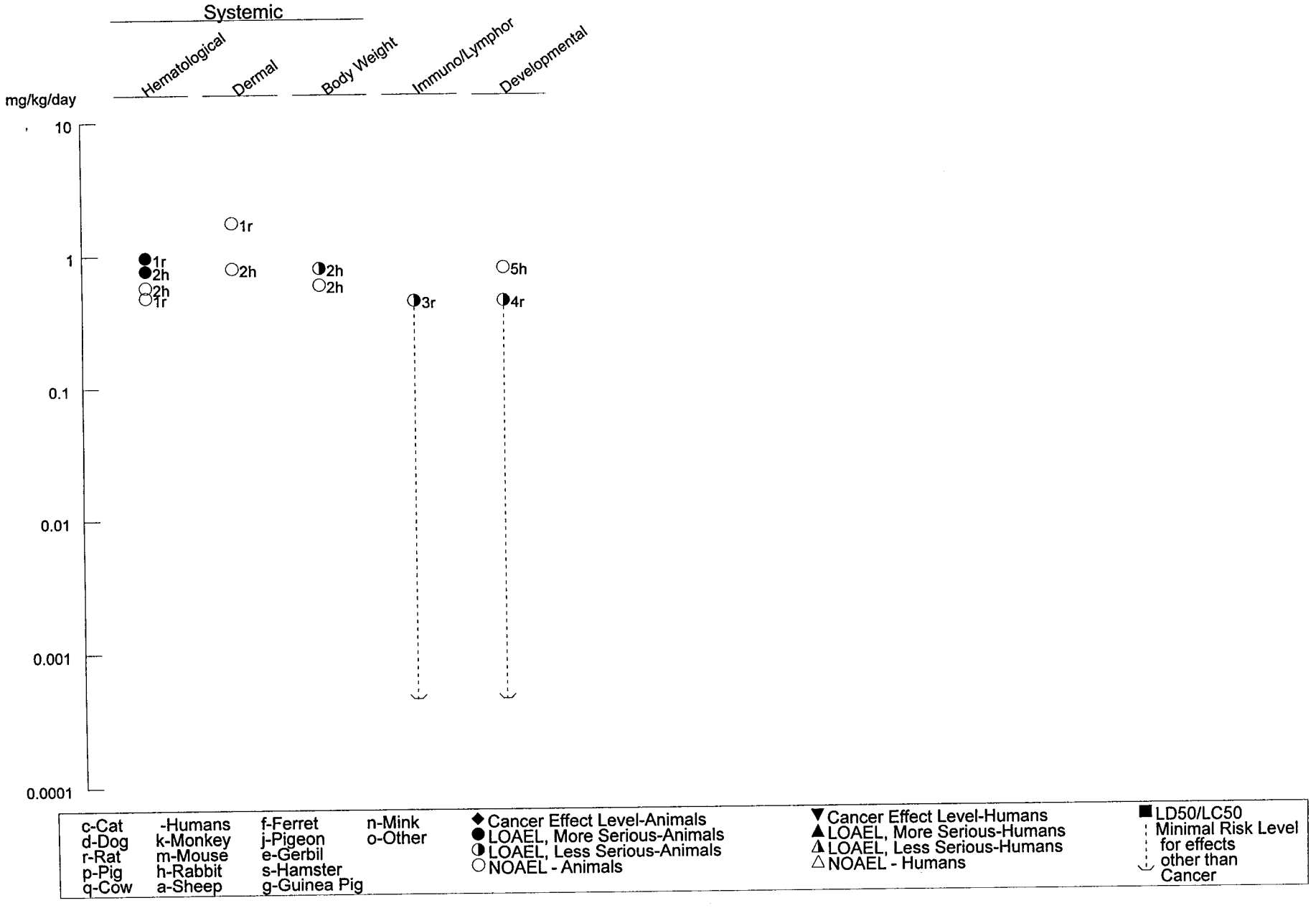

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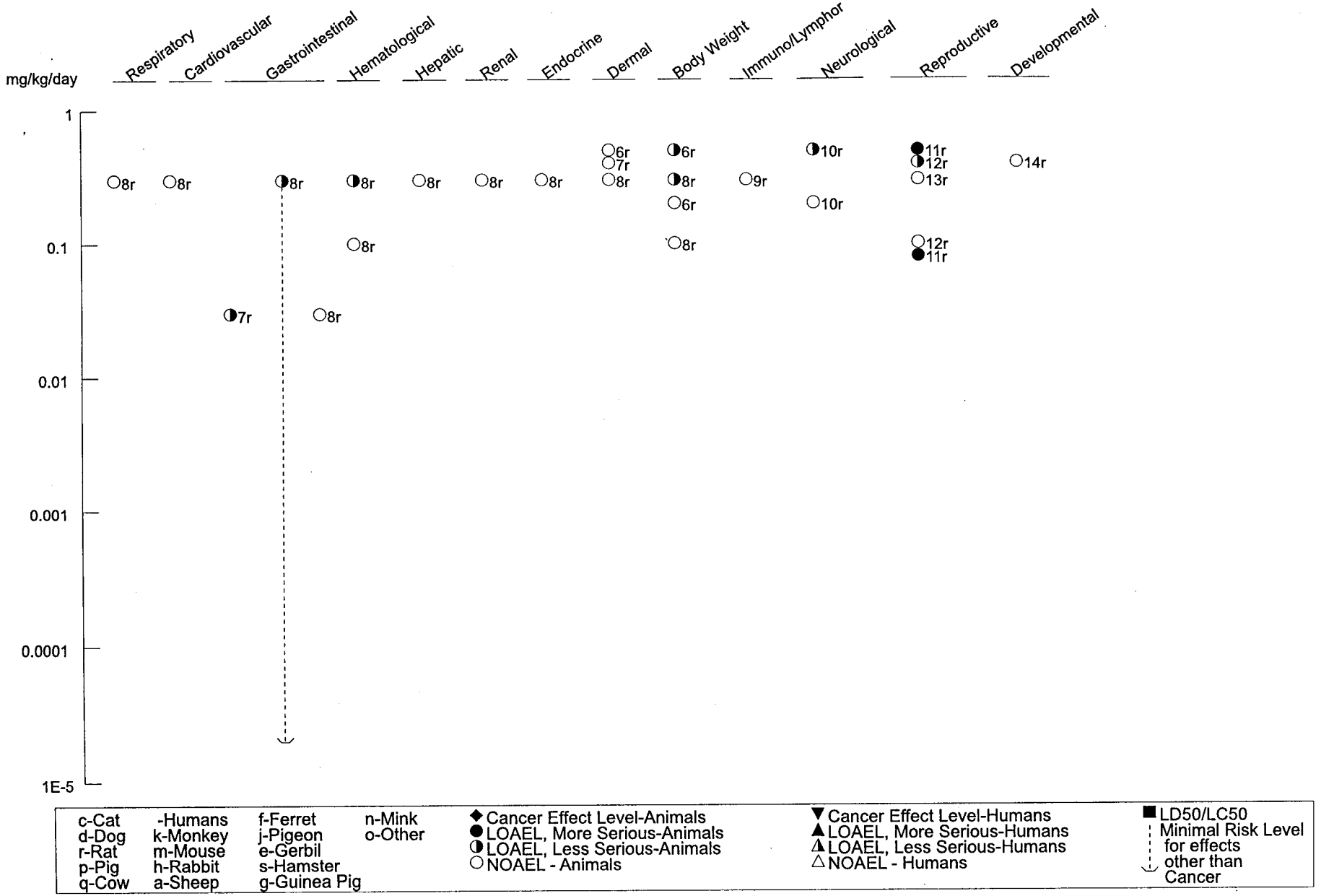
Figure 3-2. Levels of Significant Exposure to Mustard Gas - Oral (*continued*)

Intermediate (15-364 days)



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damage to the gastric mucosa at doses of 0.4 mg/kg/day and higher. Gastric ulcers were observed in 0/19 controls and in 1/18 (5.6%), 0/8, 0/18, 3/18 (16.7%), 0/8, 2/8 (25.0%), and 1/7 (14.3%) rabbits at 0.4, 0.5, 0.6, 0.8, 1.0, 2.0, and 2.5 mg/kg/day, respectively (DOA 1987b).

Rats were orally gavaged with 0.003, 0.01, 0.03, 0.1, or 0.3 mg/kg/day mustard gas for 5 days/week for 13 weeks (Sasser et al. 1996b). Microscopic examinations revealed epithelial hyperplasia of the forestomach in 10/24 (41.7%) animals (5/sex) in the highest-dose group and in one male (1/24, 4.2%) at 0.1 mg/kg. Lesions were not found in any females in the 0.1 mg/kg group or in either sex of the 0.03 mg/kg group; therefore, the forestomaches of the lower dose groups were not examined. The hyperplastic change was characterized by cellular disorganization of the basilar layer, apparent increase in mitotic activity of the basilar epithelial cells, and thickening of the epithelial layer.

Dose-related incidence and severity of lesions of the squamous epithelium of the forestomach occurred in both sexes of rats orally gavaged with 0.03, 0.1, or 0.4 mg/kg/day mustard gas for 18–21 weeks (Sasser et al. 1996a). The incidence of hyperplasia (combined F0 and F1 males and females) was 0/94 controls, 71/94 (76%; 29 male/42 female) in the low-dose groups, 89/94 (95%; 37 male/52 female) in the mid-dose groups, and 94/94 in the high-dose groups. Benign neoplasms of the forestomach (squamous papilloma) occurred in 0/94 controls, 0/94 in the low-dose groups, 8/94 (9%) in the mid-dose groups, and 10/94 (11%) in the high-dose groups.

Hematological Effects. In mated female rats, orally gavaged with 0.2, 0.4, 0.5, 0.8, 1.0, 1.6, or 2.0 mg/kg/day of mustard gas acutely on gestation days 6 through 15, maternal hematocrit values were significantly reduced by 10.8% at 0.8 mg/kg/day and 5.4% at 1.0 and 2.0 mg/kg/day (DOA 1987b). While hematocrit at 1.6 mg/kg/day was reduced, the decrease was not significant.

A dose-related decrease in maternal hematocrit was reported following acute oral administration of mustard gas on gestation days 6 through 19 in inseminated female rabbits, 0.9, 2.8, and 9.1% at 0.4, 0.6, and 0.8 mg/kg/day, respectively, with statistical significance achieved only at the highest dose (DOA 1987b).

Serum protein concentrations were significantly decreased (8.3%) only in females in the highest-dose group of rats orally gavaged with 0.003, 0.01, 0.03, 0.1, or 0.3 mg/kg/day mustard gas for 5 days/week for 13 weeks (Sasser et al. 1996b). Blood urea nitrogen (BUN) and creatinine levels and serum glutamic

oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) activities in all treated groups were comparable with controls.

Endocrine Effects. Microscopic examination of adrenals from rats orally gavaged with 0.3 mg/kg/day mustard gas 5 days/week for 13 weeks revealed no lesions (Sasser et al. 1996b).

Dermal Effects. No dermal effects were observed in rats or rabbits acutely dosed with up to 2.5 mg/kg/day of mustard gas (DOA 1987b) or following longer exposures in rats orally gavaged with 0.08–0.5 mg/kg/day mustard gas 5 days/week for 10 weeks (Sasser et al. 1993), with 0.003–0.3 mg/kg/day mustard gas 5 days/week for 13 weeks (Sasser et al. 1996b), or with 0.03–0.4 mg/kg/day mustard gas for 18–21 weeks (Sasser et al. 1996a).

Ocular Effects. Ophthalmology evaluations of rats orally gavaged with 0.003–0.3 mg/kg/day mustard gas 5 days/week for 13 weeks revealed no abnormalities (Sasser et al. 1996b).

Body Weight Effects. In mated female rats orally gavaged with 0.2, 0.4, 0.5, 0.8, 1.0, 1.6, or 2.0 mg/kg/day of mustard gas acutely on gestation days 6 through 15, a significant dose-related decrease in maternal body weights was observed by gestation day 9 at 1.0 mg/kg/day (4.7–9.1%) and 2.0 mg/kg/day (6.5–16.0%) and by gestation day 12 at 0.5 mg/kg/day (4.1–6.6%) and 1.6 mg/kg/day (9.1–16.6%) (DOA 1987b). Extragestation weight gain was also dose-related with reductions of 10, 27, 25, 29, 38, 53, and 57% measured in 0.2, 0.4, 0.5, 0.8, 1.0, 1.6, and 2.0 mg/kg/day groups, respectively, compared to concurrent controls, with statistical significance achieved at $0.4 mg/kg/day.

Inseminated female rabbits orally gavaged with 0.4–2.5 mg/kg/day of mustard gas on gestation days 6 through 19, showed a significantly decreased maternal body weight at 0.8 mg/kg/day (after gestation day 10, 5 days of exposures, 7.9–10.5% decrease) and 2.0 mg/kg/day (after gestation day 14, 9 days of exposures, 12.0–18.3% decrease), but not at 1.0 mg/kg/day (DOA 1987b).

Females in the highest-dose group of rats orally gavaged with 0.003–0.3 mg/kg/day mustard gas 5 days/week for 13 weeks weighed significantly less than controls at week 4 and during the last 5 weeks of exposure (reduced >10%) (Sasser et al. 1996b). Males in the highest-dose group weighed significantly less than controls during 6 of the weeks in the weeks 3–12 of the study period (reduced by >8%). There was no indication of a dose response in body weight in lower dose groups.

In a two-generation reproductive study of mustard gas administered intragastrically at levels of 0.03–0.4 mg/kg/day, the body weights of the F0 exposed rats were not significantly different from controls; however, the growth rate of the high-dose males tended to decline after about 7 weeks of exposure (Sasser et al. 1996a). Body weight gain beginning 1 or 2 weeks after gavaging was started (approximately 20% for males and 15–24% for females) was significantly lower ($p < 0.05$) than control values in F1 rats of both sexes born to high-dose parents. No significant dose-response in body weight occurred at the lower doses.

Rats were orally gavaged with 0.08, 0.2, or 0.5 mg/kg/day mustard gas 5 days/week for 10 weeks (Sasser et al. 1993). While body weights of female rats in all treated groups were slightly less than controls, the differences were not significant. Male body weight was significantly reduced in the high-dose group beginning at week 2 (data present graphically), whereas the weights of the lower dose groups were comparable with control.

3.2.2.3 Immunological and Lymphoreticular Effects

In mated female rats, orally gavaged with 0.2–2.5 mg/kg/day of mustard gas acutely on gestation days 6 through 15, inflamed mesenteric lymph nodes were found in 0/34 control rats, and 0/8, 4/9 (44%), 11/25 (44%), 9/9 (100%), 16/25 (64%), 6/8 (75%), 17/30 (57%), and 3/3 (100%) at 0.2, 0.4, 0.5, 0.8, 1.0, 1.6, 2.0, and 2.5 mg/kg/day, respectively (DOA 1987b).

Enlarged Peyer's patches were found in inseminated female rabbits orally gavaged with 0.4–2.5 mg/kg/day of mustard gas on gestation days 6 through 19; however, incidence was not reported (DOA 1987b).

3.2.2.4 Neurological Effects

In rats, orally gavaged with 0.08, 0.2, or 0.5 mg/kg/day mustard gas, 5 days/week for 10 weeks, drooling following dosing was observed in the highest dose group (Sasser et al. 1993).

3.2.2.5 Reproductive Effects

In teratology studies in rats and rabbits, no significant increase in the number of resorptions was reported in pregnant animals of either species orally gavaged with 0.2–2.0 mg/kg/day of mustard gas acutely on gestation days 6 through 15 (in rabbits through gestation day 19) (DOA 1987b). In rats, at the highest dose of 2.0 mg/kg/day, a significant decrease in gravid uteri weight (16%) and sex ratio (46.2% males) occurred.

Microscopic examination of testes from rats orally gavaged with 0.3 mg/kg/day mustard gas 5 days/week for 13 weeks revealed no lesions (Sasser et al. 1996b).

Reproductive performance and fertility in male or female rats through two consecutive generations were studied following exposure to mustard gas via intragastric administration at levels of 0.03, 0.1, and 0.4 mg/kg/day (Sasser et al. 1996a). Microscopic examination of the reproductive organs revealed no evidence of treatment-related effects. The only statistically significant birth parameter difference between treated and control groups was an increase in the sex ratio (fraction of males) of the high-dose F0 offspring.

In a dominant lethal study of mustard gas, rats were orally gavaged with 0.08, 0.2, or 0.5 mg/kg/day mustard gas 5 days/week for 10 weeks (Sasser et al. 1993). In female dominant lethality experiments (treated or untreated males were mated with treated females), the overall mean pregnancy rate in treatment groups was 86%; treatment means ranged from 70 to 100%, with no significant differences between treatment groups. Reproductive performance indicators (number of live or dead implants, resorptions, and preimplantation losses) in treated female rats mated to treated or nontreated males were not significantly different from controls. In male dominant lethality experiments (treated males were mated with untreated females), the overall mean pregnancy rate in treatment groups was 91%; treatment means ranged from 65 to 100%, with no significant differences between treatment groups. There was no indication of a dose relationship with the number of live implants. In the highest exposure group, the mean number of total and early resorptions per litter was significantly greater than control during the 2nd and 3rd postexposure weeks. The number of total and late resorptions in the mid-dose group was also greater than controls during the 3rd postexposure week. Preimplantation losses in the mid- and high-dose groups were also significantly elevated during the 2nd postexposure week. High-dose male sperm morphology data at all postexposure sampling times, 0, 5, and 12 weeks, showed a statistically significant

decrease in the percentage of normal sperm. Blunthook and banana-shaped sperm heads were observed at 0, 5, and 12 weeks, whereas amorphous and short head abnormalities were observed only at 5 and 12 weeks. Overall, there was a total 2-fold increase in abnormal sperm heads in high-dose mustard gas-treated males. In summary, female fertility was not affected by these mustard gas exposures; however, a male dominant lethal effect was demonstrated at the mid and high doses of mustard gas.

3.2.2.6 Developmental Effects

Teratology studies were conducted in rats and rabbits by DOA (1987b). Rats were exposed to 0.5–2.0 mg/kg of mustard gas by gastric intubation from 6 to 15 days of gestation. Fetal body weight was significantly decreased (6–7%) from control in rat litters exposed to doses of 1.0 and 2.0 mg/kg/day; no clear dose-relation was evident. The sex ratio (percent males) was significantly lower than control at 2.0 mg/kg/day (46.2 vs. 51.0%). Placental weight was significantly reduced (8.4%) at 2.0 mg/kg/day. Supernumerary ribs were found in 9/299 (3%) fetuses of one litter in the 2.0 mg/kg/day group, while this anomaly was not found in any of the fetuses in the lower dose or control groups. The incidence of reduced ossification of the vertebrae and/or sternebrae in all treated groups was significantly higher than control when individual pup data were compared but not with litter comparisons, 42/272 (15%) in controls, 51/229 (22%) at 0.5 mg/kg/day, 76/315 (24%) at 1.0 mg/kg/day, and 72/299 (24%) at 2.0 mg/kg/day. All fetal effects in rats occurred at doses that also produced maternal toxicity. In rabbits exposed to 0.4–0.8 mg/kg of mustard gas between 6 and 19 days of gestation, the only effect of mustard gas on fetuses was a significant reduction in fetal body weight (38%), which occurred at 2.0 mg/kg/day, a dose that also produced maternal toxicity.

Developmental effects in male or female rats through two consecutive generations were studied following exposure to mustard gas via intragastric administration at levels of 0.03, 0.1, and 0.4 mg/kg/day (Sasser et al. 1996a). Although, not significantly different, litter weights and number of pups per litter tended to decrease in both F1 and F2 generations at the highest exposure level.

3.2.2.7 Cancer

No studies were located regarding cancer in humans or animals after oral exposure to mustard gas.

3.2.3 Dermal Exposure

3.2.3.1 Death

In France, two children died after a 40-year-old mustard gas shell accidentally exploded spraying the liquid onto their skin and clothing (Heully et al. 1956). Two fishermen died from handling mustard gas bombs disposed of in the Baltic Sea which became caught in their nets (Aasted et al. 1985; Jorgensen et al. 1985). Other surviving fishermen suffered skin lesions, erythema, blistering, and eye lesions. In humans, the LD_{50} for skin exposure is estimated to be 100 mg/kg (SBCCOM 1999). LD_{50} values in animals for mustard gas administered topically range from 9 to 100 mg/kg (Dacre et al. 1995). Of the species studied (rat, mouse, dog, rabbit, guinea pig, and goat), the rat was the most sensitive, with a dermal LD_{50} of 9 mg/kg.

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, musculoskeletal, renal, or body weight effects in humans or animals after dermal exposure to mustard gas.

Gastrointestinal Effects. Volunteers who were wearing respirators and who were exposed to unspecified levels of mustard gas vapors and liquids had skin burns, but also complained of nausea, vomiting, anorexia, abdominal pain, diarrhea, headache, and lassitude (Sinclair 1948). These signs could have been primary effects of the mustard gas on the rapidly dividing cells of the gastrointestinal epithelium, secondary effects from the skin burns, or psychological effects not related to the mustard gas exposure at all.

In a study designed to determine lethal dermal doses, rats stopped eating and drinking, had diarrhea, and lost weight prior to death (Young 1947).

Hematological Effects. Mustard gas was topically applied a single time at doses of 3.88, 7.75, or 15.5 mg/kg to the shaved backs of Balb/c mice (Venkateswaran et al. 1994). A reduction in lymphocytes was noted. Hematology revealed a significant dose-related increase in packed cell volume (10–16%). The increase in hemoglobin concentration was also dose-related and significant at mid and high doses (13–19%).

Hepatic Effects. Mustard gas was topically applied a single time at doses of 3.88, 7.75, or 15.5 mg/kg to the shaved backs of Balb/c mice (Venkateswaran et al. 1994). A dose-related decrease in liver weight was observed, with a significant reduction of 14% measured a the high dose.

A single dose of 51.3 mg/kg (1 LD_{50}) of neat mustard gas was applied to the hair-clipped backs of male guinea pigs (Chauhan and Murty 1997). At 24 hours after exposure, microscopic examinations of the liver revealed fatty degeneration accompanied by infiltration with red blood cells, lipidolysis, and distortion of cell structure. At 3 days postexposure, infiltration with macrophages was observed in addition to the above alterations. Liver injury was also indicated by increases in blood enzymes, glutamic oxaloacetic transaminase (GOT), and glutamic pyruvate transaminase (GPT). Both enzymes increased after exposure reaching maximum levels of nearly twice control values at 3 days, and returned toward normal levels at 6 days postexposure. The GOT recovery was slower than GPT as the 6-day level, while submaximal, was still significantly elevated (33%) above control.

Endocrine Effects. Mustard gas was topically applied a single time at doses of 3.88, 7.75, or 15.5 mg/kg to the shaved backs of Balb/c mice (Venkateswaran et al. 1994). A dose-related increase in adrenal weight was observed, significant at the mid and high doses (25–57%).

Dermal Effects. The severity of cutaneous injury is dose-dependent and is directly related to the mustard gas alkylation levels in skin (Papirmeister 1993). Vesication and acute tissue injury only occur at mustard gas alkylation levels much higher than those needed to produce genotoxic effects. Tissue injury does not develop when low, therapeutically effective doses of mustard gas are used to control the hyperproliferation of psoratic keratinocytes.

Mustard gas is more harmful to the skin on hot, humid days, or in tropical climates (Sulzberger et al. 1947). SBCCOM (1999) reports a maximum safe Ct of 5 mg-minute/m^3 for human skin exposure. The ICt_{50} (estimated concentration-exposure time period product incapacitating to 50% of exposed individuals) for human skin exposure is dependent on temperature, 2,000 mg-minute/m^3 at 70–80 EF (humid environment) and 1,000 mg-minute/m^3 at 90 EF (dry environment) (SBCCOM 1999).

When mustard gas gets on human skin, it causes erythema, itching, and blisters. These reactions are usually delayed by at least several hours, up to 48 hours (Jakubowski et al. 2000; Renshaw 1946; Smith et al. 1919). Australian soldiers, who were wearing respirators, volunteered to be exposed to skin contact with mustard gas during World War I. They had erythema on the exposed areas, and skin burns on the genitalia (Sinclair 1948, 1950). They also suffered from nausea and vomiting, but this may be secondary to the skin burns. Other subjective complaints, such as headache and lassitude, could be secondary to the burns, primary effects of the mustard gas, or due to other causes altogether. Men who were exposed to mustard gas from leaking artillery shells picked up by fishing vessels off the coast of Denmark showed inflamed skin, blisters, eye irritation, and transient blindness (Wulf et al. 1985). Army volunteers exposed to mustard gas had skin burns, but no increased incidence of skin cancer or other systemic effects (NRC 1985).

There is a case report of an accidental exposure to mustard gas in the laboratory (Jakubowski et al. 2000). A chemist was measuring what was believed to be a completely innocuous liquid mixture using a liquid flashpoint tester. The apparatus, which was in an approved chemical laboratory hood, overheated, causing vigorous vapor and aerosol generation from the sample, to which the subject was exposed primarily by skin contact. Personal decontamination was delayed for 5–10 minutes after exposure. Subsequent analysis showed that the liquid contained more than 5% mustard gas. The subject noticed no effects until about 9 hours after exposure, at which time, burning was experienced on his arms, hands, face, and neck. He applied a topical anesthetic-antiseptic before bed and awoke the next morning with blisters on his hands and arms. Mustard gas was not detected in the blister fluid, but thiodiglycol, a metabolite of mustard gas, was detected in his urine for 13 days following exposure.

A review of the literature prior to 1950 indicates that drops containing 0.1% or more mustard gas can cause skin blisters on humans (Sulzberger et al. 1947). The amounts applied during these studies could not be well quantified. Humans show varying degrees of sensitivity to mustard gas (Renshaw 1946; Sulzberger et al. 1947). In particular, people with fair skin are more sensitive than those with dark skin. These reports also indicate that individuals with previous exposure are more sensitive to the dermal effects of mustard gas.

A group of patients, including a subgroup of 14 children and teenagers (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years), were examined in a hospital in Iran 18–24 hours following exposure to mustard gas from air bombs during the Iran-Iraq War (Momeni and Aminjavaheri 1994).

Cutaneous effects included erythema (in 94% of patients), itching (71%), bulla (71%), ulceration (64%), hyperpigmentation (50%), and hypopigmentation (21%). Burning sensation (in 71% of patients) and pain (36%) were also noted. Skin lesions first appeared 4–18 hours after exposure, accompanied by an itching and burning sensation, especially over the face and neck. Thereafter, the patients developed erythema and gradually, after 20–30 hours, blisters. Most of the lesions in children developed of the face (79%), followed by genital (43%), thoracic (21%), trunkal (14%), and axillar lesions (14%). No direct relation was found between sex of the individual and the site of the lesions. The time of onset of mustard gas manifestations in children was shorter (4–18 hours) and the severity of the lesions higher than in adults (8–24 hours), possibly due to more delicate skin and epithelial tissues. Genital lesions were less frequent in children and teenagers (42%) than adults (70%); however, even within the group of children, the incidence and severity of genital lesions increased with age. Other skin lesions had no apparent age-relation.

Mustard gas applied to the skin of rats produced local edema, which subsided after 3 days (Young 1947). In mice, rabbits, and guinea pigs, mustard gas produced vascular leakage, leukocytic infiltration, and slow death of basal epidermal cells; this damage reached its peak 1–3 days after application (Vogt et al. 1984; Chauhan et al. 1993a, 1993b, 1995, 1996). Healing occurred within 10 days. Suckling rats (which had not yet grown hair) developed inflammatory changes and epidermal thickening after dermal exposure to mustard gas for 1–15 minutes (McAdams 1956). This damage was evident 1–7 days postexposure. Blisters did not develop, but the basal cells were destroyed.

Mustard gas was topically applied a single time at doses of 3.88, 7.75, or 15.5 mg/kg to the shaved backs of Balb/c mice (Venkateswaran et al. 1994). Mild skin lesions first appeared on postexposure day 4. Lesions progressed to severe, with fluid loss, on postexposure day 7.

Ocular Effects. The eyes are more sensitive to mustard gas than the skin or respiratory tract. In humans, an ICt_{50} (estimated concentration-exposure time period product incapacitating to 50% of exposed individuals) and a maximum safe Ct for eye exposure are 200 and 2 mg-minute/m^3, respectively (SBCCOM 1999). The damage may vary from mild conjunctivitis to severe corneal involvement with dense opacification, ulceration, and vascularization. In men, mild reddening of the eyes, but no incapacitation, resulted at 70 mg-minute/m^3 (McNamara et al. 1975). At 90 mg-minute/m^3, the eyes were marginally incapacitated with grittiness, photophobia, lacrimation, discharges, and staining, all of which disappeared within 4 days after exposure. Eye pain and spasmodic blinking are other reported ocular

effects. A temporary loss of vision occurred in men exposed to 100 or 144 mg-minute/m^3. In seven men who were accidentally exposed to estimated doses of 200–300 mg-minute/m^3, severe to total impairment of vision resulted in all (McNamara et al. 1975). Eyes exposed to mustard gas can also result in delayed reactions that are manifested as delayed relapsing keratitis (Amalric et al. 1965; Dahl et al. 1985; Mann 1944).

A group of patients, including a subgroup of 14 children and teenagers (9 boys, aged 9 months to 14 years; 5 girls, aged 13 months to 9 years), were examined in a hospital in Iran 18–24 hours following exposure to mustard gas from air bombs during the Iran-Iraq War (Momeni and Aminjavaheri 1994). Ocular effects of conjunctivitis and photophobia were most prevalent, each occurring in 93% of the children, with lower incidences of edema of the eyelids (57%), closure of the eyes (43%), keratitis (43%), blepharospasm (43%), subconjunctival hemorrhage (14%), and corneal ulcer in one child (7%). Burning sensation (71%) and pain (36%) were also noted. The burning sensation in their eyes developed 3–4 hours after exposure and was followed by photophobia and conjunctivitis. Ocular effects had higher occurrences in children than in adults.

The eyes of dogs that were exposed to 0.1 mg/m^3 (0.015 ppm) of mustard gas for 16 weeks showed corneal opacity, vascularization, and granulation (McNamara et al. 1975). Similar results were reported by Winternitz (1920), who exposed dogs acutely to unspecified levels of mustard gas.

The retinas of rats sacrificed 24 hours after injected subcutaneous injection in the dorsal area with 10 µL of undiluted radiolabeled mustard gas showed edematous swelling of the inner layers. Cell degenerative changes included dense cytoplasm, enlarged mitochondria, and Golgi apparatus. Rats sacrificed at 48 hours after injection had highly disorganized and vacuolated outer segment membranes and the choroid vessels contained large clusters of activated platelets (Klain et al. 1991).

Mustard gas was topically applied a single time at doses of 3.88, 7.75, or 15.5 mg/kg to the shaved backs of Balb/c mice (16/group/dose) (Venkateswaran et al. 1994). Reduced food consumption was noted in the high-dose group. A progressive dose dependent fall in body weight beginning 3–5 days after exposure was found; the decrease was significant at the mid and high doses, 11 and 27%, respectively.

Guinea pigs treated with a single dose of 51.3 mg/kg (1 LD_{50}) of neat mustard gas applied to their hair-clipped backs showed a gradual loss of weight up to 35% on postexposure day 6 (Chauhan and Murty 1997).

3.2.3.3 Immunological and Lymphoreticular Effects

Mustard gas was topically applied a single time at doses of 3.88, 7.75, or 15.5 mg/kg to the shaved backs of Balb/c mice (16/group/dose) (Venkateswaran et al. 1994). Mustard gas produced a significant dose-related decrease in the weight of the spleen (12–59%), and peripheral (12–44%) and mesenteric lymph nodes (significant only at high dose, 18%). Incidence and severity of histological changes in the thymus and spleen were also dose-related. Spleen histopathology included hypocellularity, atrophy of the lymphoid follicles, degeneration of germinal centers, and red pulp infiltrated with macrophages. The cortex and medulla regions of the thymus showed atrophy and hypocellularity. Red blood cells replaced cortical thymocytes with severe atrophy. A significant dose-related decrease in the cellularity of the spleen (24–45%) was measured. A dose-related decrease in the cellularity of the thymus was also found, significant at the mid and high doses (36–42%).

Cameron et al. (1946), after observing damage to the cervical lymph nodes and lymphoid tissue throughout the body in rabbits and monkeys that had undergone tracheal cannulation and were exposed to nominal chamber concentrations of mustard gas ranging from 30 to 350 mg/m^3 (5–54 ppm), administered mustard gas to animal skin and observed identical changes to the lymph tissue, suggesting that lymphoid tissue damage may be due to systemic absorption. Only a general discussion, lacking experimental details, was reported.

3.2.3.4 Neurological Effects

Chronic and/or late neurological symptoms in the skin after exposure to sulfur mustard were studied in five patients exposed to mustard gas during battlefield operations in the Middle East and five fishermen accidentally exposed to sulfur mustard by pulling shells leaking the chemical agent aboard their fishing vessels. All 10 patients (100%) suffered from neuropathic pain or other deafferentation symptoms, suggesting persistent damage to the afferent nerve system as a frequent complication in persons exposed to mustard gas (Thomsen et al. 1998).

Guinea pigs treated with a single dose of 51.3 mg/kg (1 LD_{50}) of neat mustard gas applied to their hair-clipped backs became sedated 1 day after exposure (Chauhan and Murty 1997).

No studies were located regarding the following heath effects in humans or animals after dermal exposure to mustard gas:

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

Five cases of Bowen's disease (a type of skin cancer) were studied among 488 former workers of a Japanese poison gas factory (Inada et al. 1978). Workers were manufacturing mustard gas for 3–15 years and the diagnosis was made 31–41 years after exposure. These workers also suffered from acute dermatitis, conjunctivitis, bronchitis, and hyperkeratotic skin eruptions. The occurrence of Bowen's disease, Bowen's carcinoma, basal cell carcinomas, and carcinoma spinocellular has also been reported in survivors of the dismantling of the "Heeres-Munitionsanstalt St. Georgen" who were exposed to poisonous gases including mustard gas by skin contact and inhalation (Klehr 1984).

No studies were located regarding cancer in animals after dermal exposure to mustard gas, although animals exposed to mustard gas in the air could have also had skin exposure. Cancer in these animals is discussed in Section 3.2.1.7 above.

3.2.4 Other Routes of Exposure

Several animals studies indicate effects of mustard gas on the hemopoietic system following intravenous or subcutaneous administration of mustard gas. Single intravenous injection of 0.5 mg/kg of mustard gas in young male rats caused degenerative damage to the spleen, thymus, and bone marrow (Kindred 1947). This was also observed in rats, mice, rabbits, and dogs following a subcutaneous injection of 3 mg/kg of mustard gas (Graef et al. 1948). Within 12 hours of injection, granulocytosis was observed, followed by leukopenia. In addition to hemopoietic tissue damage, injury to the testes with inhibition of spermatogenesis were also observed. Subcutaneous injection of 0.625 mg/kg of mustard gas in male rats caused injury to the thymus (Cataline et al. 1971). An intraperitoneal injection of 15 mg/kg of mustard

gas depressed the activity of bone marrow cells of the femur in mice (Friedberg et al. 1983). Parental administration of mustard gas to laboratory animals resulted in death due to systemic intoxication, with little or no involvement of the eyes or skin. Damage to the lungs is seen with intravenous administration, but not other parenteral routes (Anslow and Houch 1946).

A significant dose-related reduction in spleen cell number was measured in mice 7 days after intraperitoneal injection with mustard gas (23% at 5 mg/kg and 49% at 10 mg/kg) (Coutelier et al. 1991). Sv female mice (5–9/group) were injected intraperitoneally with a single dose of 2, 5, or 10 mg/kg mustard gas (>90% purity) in a 1% isopropanol solution in saline. A 26% increase in spleen T-lymphocytes and a 44% decrease in B-lymphocytes was measured 7 days following injection with 10 mg/kg of mustard gas. B- and T-lymphocyte function, as assayed by *in vitro* thymidine incorporation and/or immunoglobulin secretion, was not significantly affected by mustard gas.

Mustard gas, administered to guinea pigs by intratracheal injection, induced a 3-fold increase in respiratory system resistance, accompanied by a significant decrease in compliance (Calvet et al. 1993). Capsaicin-sensitive nerves do not have primary involvement in the acute respiratory effects of mustard gas as preteatment with capsaicin did not prevent acute effects. Fourteen days after exposure, substance P induced concentration-dependent bronchoconstriction in guinea pigs, and tracheal epithelium neutral endopeptidase (NEP), the main enzyme that degrades tachykinins, was reduced significantly (40%) from the control level. While hyper-responsiveness to substance P has been attributed to a decrease in the tracheal activity of NEP and corresponding increase in tachykinins, this hypothesis was not upheld, as pretreatment with phosphoramidon, a NEP inhibitor, only increased mustard gas-induce hypersensitivity to substance P. Phosphoramidon administered prior to vehicle control ethanol also increased sensitivity to substance P.

3.3 GENOTOXICITY

Low doses of mustard gas can inhibit cell division due to its ability to cross-link complementary strands of DNA or produce mutagenesis, which may be caused by replication errors or misrepair (Papirmeister 1993). DNA is the most functionally sensitive target of mustard gas in cells. Men who were exposed to mustard gas from leaking shells picked up by fishing vessels showed increased sister chromatid exchanges in their lymphocytes (Wulf et al. 1985). However, the offspring of workers exposed to mustard gas in a Japanese factory showed no increases in diseases that would be indicative of genetic

damage (Yamakido et al. 1985). Mustard gas induced dose-related interstrand cross-links in the DNA of rat epidermal keratinocytes in primary monolayer cultures, synchronized at the G1/S boundary or in the G1 phase of the cell cycle (Lin et al. 1996a). At 24 hours postexposure, the level of cross-links in cells exposed at the G1 phase had not decreased significantly and was still dose-dependent. However, at 24-hours postexposure, cells exposed in the G1 phase showed a major decrease in cross-links.

DNA extracted from white blood cells of human blood and exposed to [14C]-labeled mustard gas *in vitro* was shown to contain the DNA adduct 7-(2-hydroxyethylthio-ethyl) guanine (Ludlum et al. 1994). Mustard gas alkylation has been shown to induce effects on transcriptional processes (Masta et al. 1996). Gel mobility shift analysis showed that mustard gas alkylation of the lac UV5 promoter increased the stability of the promoter-RNA polymerase binary complex. Following formation of the initiation complex and addition of elongation nucleotides, approximately 45% of the RNA polymerase in the initiated complex remained associated with the alkylated promoter, compared to only 7% remaining associated with the unalkylated promoter. For the RNA polymerase able to escape the initiation complex, mustard gas alkylation of the DNA template resulted in the production of truncated transcripts. Analysis of the truncated transcripts revealed that mustard gas alkylates DNA preferentially at 5'-AA, 5'-GG, and 5'-GNC sequences on the DNA template strand.

Mustard gas has been shown to affect the cell cycle and DNA synthesis in epidermal basal keratinocytes. When primary keratinocytes were exposed to mustard gas in different phases of the cell cycle, cells in the S phase were more sensitive to mustard gas than cells in the other phases (Lin et al. 1996b). Keratinocytes exposed to 1 µM mustard gas at the G1/S boundary exhibited a prolongation of the S phase and a block in the G2 phase. When these cells were exposed to 10 or 50 µM mustard gas, they did not enter the S phase for up to 12 hours and the incorporation of thymidine into DNA was inhibited, suggesting that the blocks in the G2 and G1 phases relate to the cytotoxic effect of mustard gas.

Mustard gas at concentrations of 0.5 and 0.1 mM produced single strand breaks in bacteriophage lambda DNA (Venkateswaran et al. 1994), which were prevented by the presence of magnesium ions in the reaction mixture. The authors proposed that the degradation of lambda DNA by its interaction with mustard gas may be caused by the breakage of phosphodiester backbone of DNA via the formation of an intermediate phosphotriester bond.

Mustard gas-induced DNA damage in primary monolayer cultures of rat cutaneous keratinocytes was assessed with the nucleoid sedimentation assay (Ribeiro et al. 1991). Within 1 hour of exposure to as little as 0.1 µM mustard gas, the structural integrity of cellular DNA was compromised. The gross structural integrity of the DNA in cells exposed to as much as 5 µM mustard gas was completely restored within the first 22 hours following the exposure. However, this repair process appeared to be inefficient, since a depression of thymidine incorporation into DNA and a significant loss of DNA were exhibited in exposed cultures as long as 72 hours after the initial exposure.

Fan and Bernstein (1991) evaluated the possible effects of mustard gas on the repair of mismatched bases in the DNA of African green monkey kidney (AGMK) cells transfected with heteroduplex (ht) DNA, formed between two temperature-sensitive mutants of SV40 virus, tsA239 and tsA255, each having a different point mutation in the gene for large T antigen. In order for the cells to produce wild type SV40 DNA at a nonpermissive temperature, repair of at least one of the two mismatches in the DNA had to occur. As the concentration of mustard gas was increased, a proportionally longer delay in the appearance of wild type DNA was observed in treated cells transfected with ht DNA as compared with cultures exposed to solvent alone and then transfected with ht DNA. This effect did not occur in mustard gas exposed AGMK cells transfected with wild type DNA, suggesting that mustard gas does affect mismatched base repair.

A variety of *in vitro* assays, summarized in Table 3-3, provide positive genotoxicity results. These data support the few human data on *in vivo* exposures to this compound. The *in vitro* data from both prokaryotic organisms (*Salmonella typhimurium* and *Escherichia coli*) and eukaryotic organisms (HeLa cells, mouse lymphoma, mouse L cells, rat lymphosarcoma) all support a mechanism of DNA alkylation leading to cross-link formation, inhibition of DNA synthesis and repair, point mutation, and chromosome and chromatid aberration formation.

There are also data from *Drosophila* experiments in which sulfur mustard was injected into male flies, leading to sex-linked lethal mutations and point mutations at one of the loci affecting bristle formation (Auerbach 1946; Fahmy and Fahmy 1971, 1972). Mustard gas has also been shown to be a micronucleus-inducing agent to the mouse bone marrow (Ashby et al. 1991). All of these data are consistent with this agent being a powerful genotoxicant, which supports the recognized carcinogenicity of mustard gas.

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Table 3-3. Genotoxicity of Mustard Gas *In Vitro*

Species (test system)	End point	Results: With activation	Results: Without activation	Reference
Prokaryotic organisms:				
Escherichia coli	DNA interstrand crosslinks	+	No data	Venitt 1968
E. coli	DNA recombination repair inhibition	+	+	Ichinotsubo et al. 1977
Salmonella typhimurium	Gene mutation	+	+	Ichinotsubo et al. 1977
S. typhimurium	Gene mutation	+	+	Ashby et al. 1991
Eukaryotic organisms:				
Fungi:				
Saccharomyces cerevisiae	DNA alkylation	+	No data	Kircher and Brendel 1983
Human HeLa cells in culture	DNA crosslinking	+	No data	Ball and Roberts 1971/72
Mouse lymphoma cells	Gene mutation	+	No data	Capizzi et al. 1974
Mouse lymphoma cells	Chromosomal and chromatid aberrations	+	No data	Scott et al. 1974
Rat lymphosarcoma cells	Chromosomal and chromatid aberrations	+	No data	Scott et al. 1974
Rat lymphosarcoma cells	DNA replication repair inhibition	+	No data	Scott et al. 1974
Mouse fibroblasts, L-strain	Inhibition of DNA synthesis	+	No data	Walker and Thatcher 1968

+ = positive result; DNA = deoxyribonucleic acid

Transcription, translation, and enzyme catalysis, cellular activities that are dependent on biological entities of much lower molecular size than chromosomal DNA, are much less sensitive to mustard gas (Papirmeister 1993). Thus, cells that are prevented from synthesizing DNA continue to generate energy and synthesize RNA and protein. As a result of this unbalanced metabolism, cells may enlarge, differentiate, or be induced to synthesize high levels of certain proteins. While some of these proteins may protect cells, others may hasten cell death.

Vesication and acute tissue injury only occur at mustard gas alkylation levels much higher than those needed to produce genotoxic effects. Tissue injury does not develop when low, therapeutically effective doses of mustard gas are used to control the hyperproliferation of psoratic keratinocytes. Therefore, it is likely that additional mechanisms other those related to genotoxicity are responsible for acute toxicity of mustard gas.

3.4 TOXICOKINETICS

There is a substantial toxicokinetic database for intravenous and intraperitoneal routes of mustard gas exposure in animals. While these data are useful, there is evidence to suggest that this information does not mimic the scenario resulting from field or accidental conditions that expose humans to mustard gas by absorption from the skin, or by the lung or eyes. Mustard gas tissue distribution data from an Iranian soldier who died 7 days after inhalation and/or dermal exposure to mustard gas indicated distribution: brain > kidney > liver > spleen > lung (Drasch et al. 1987), whereas radiolabel concentration data in rats 4 days after an intravenous injection of radiolabeled mustard gas indicate a different distribution pattern to these organs: kidney > lung > liver > spleen > brain (Maisonneuve et al. 1994). While the difference could be due to measurement methods, species variations, or postexposure time, the route of exposure appears to be a significant toxicokinetic factor.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Since mustard gas can be found in human tissues following exposure through the air, it can apparently be absorbed through the lungs or skin (Drasch et al. 1987). Analyses of the blood of hairless guinea pigs

after 8-minute nose-only exposure to 300 mg/m^3 (46 ppm) of mustard gas indicated that the concentration of mustard gas in blood peaked within 5 minutes after exposure (Langenberg et al. 1998).

In rabbits and monkeys that had undergone tracheal cannulation and were exposed to nominal chamber concentrations of 40, 100, and 500 mg/m^3 of mustard gas, about 15% of the dose was recovered, indicating that 85% was absorbed through the mucous membrane of the nose (Cameron et al. 1946).

3.4.1.2 Oral Exposure

No studies were located regarding absorption in humans or animals after oral exposure to mustard gas.

3.4.1.3 Dermal Exposure

Since mustard gas can be found in bodily tissues of humans following exposure through the air, it is apparently absorbed through the lungs or through the skin (Drasch et al. 1987). When applied to human skin, most of the mustard gas evaporates (Smith et al. 1919). Some of the vapors can be absorbed into the skin, with the majority of this being absorbed into the blood stream (Cullumbine 1946, 1947; Nagy et al. 1946; Renshaw 1946). Renshaw (1946) reported that 80% of unoccluded, topically-applied mustard gas evaporates from the skin and the remaining fraction penetrates the skin. This finding has been confirmed in studies of human foreskin grafted onto athymic mice (Papirmeister et al. 1984a, 1984b).

The absorption of mustard gas through the cornea was demonstrated in guinea pigs (Klain et al. 1991). Following 30 minutes after a single topical application of 5 µL of radiolabeled mustard gas to the cornea of guinea pigs, radioactivity was detected in kidney, liver, lung, adipose tissue, adrenals, plasma, and muscle.

Hambrook et al. (1993) reported that after a 6-hour cutaneous exposure with occlusion, >90% of applied dose was absorbed in rat skin. The initial rate of uptake, within 60 minutes of loading, increased linearly with applied dosage in the range of 3–605 µg/cm^2 (0.2–3.8 µmol/cm^2), and reached a maximum of approximately 7 µg/cm^2/minute (0.044 µmol/cm^2/minute) at a dosage of 955 µg/cm^2 (6 µmol/cm^2) (Hambrook et al. 1993). A range of skin-retention fractions from 10 to 50% have been reported (Cullumbine 1947; Hambrook et al. 1992; Renshaw 1946), while the remaining mustard gas is absorbed systemically. The rate of penetration of mustard gas into human skin was estimated in the range of

1–4 µg/cm^2/minute (0.006–0.025 µmol/cm^2/minute) (Renshaw 1946). Skin penetration of mustard gas is proportional to its temperature (Nagy et al. 1946). Some authors have suggested that the mustard gas is absorbed into the skin by passing into the sweat glands (Smith et al. 1919).

3.4.1.4 Other Routes of Exposure

No studies were located regarding absorption in humans or animals after exposure to mustard gas by routes other than inhalation and dermal.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

Analyses of body fluids and tissues of an Iranian soldier who died 7 days after exposure to mustard gas (by inhalation and/or dermal routes) indicated that mustard gas was distributed to cerebrospinal fluid and, in order of decreasing concentrations, fat (from thigh), brain, abdominal skin, kidney, muscle, liver, spleen, and lung (Drasch et al. 1987). No mustard gas was found in the urine of this patient. Analyses of the blood of hairless guinea pigs after 8-minute nose-only exposure to 300 mg/m^3 (46 ppm; 2,400 mg-minute/m^3) of mustard gas indicated that the concentration of mustard gas in blood peaked within 5 minutes after exposure, dropped to about 50% of peak at 30 minutes, and gradually increased again to about 60% of peak concentration at 4 hours after exposure (Langenberg et al. 1998). Evidence of tissue mustard gas DNA adducts in hairless guinea pigs at 4 hours after 5-minute nose-only exposure to 160 mg/m^3 (25 ppm; 800 mg-minute/m^3) of mustard gas indicates absorption and/or distribution to nasal epithelium, nasopharynx, larynx, carina, and lung (Langenberg et al. 1998). Mustard gas DNA adducts found in the lung, spleen, and bone marrow in the same species after 8-minute nose-only exposure to 300 mg/m^3 (46 ppm; 2,400 mg-minute/m^3) of mustard gas indicates distribution to these tissues (Langenberg et al. 1998).

3.4.2.2 Oral Exposure

No studies were located regarding distribution in humans or animals after oral exposure to mustard gas.

3.4.2.3 Dermal Exposure

Analyses of body fluids and tissues of an Iranian soldier who died 7 days after exposure to mustard gas (by inhalation and/or dermal routes) indicated that mustard gas was distributed to cerebrospinal fluid and, in order of decreasing concentrations, fat (from thigh), brain, abdominal skin, kidney, muscle, liver, spleen, and lung (Drasch et al. 1987). No mustard gas was found in the urine of this patient. Older reports have stated that mustard gas is distributed to most tissues in humans (Cullumbine 1947). Hambrook et al. (1993) reported that after a 6-hour cutaneous exposure to radiolabeled mustard gas with occlusion, 10–23% of absorbed radiolabel dose was retained in rat skin, with range of 3–7% detected in blood. At the end of the 6-hour application, when the level of radiolabel in the blood reached a maximum, greater than 90% of the red cell radiolabel activity was found in the cell contents, with the remaining in the red cell membranes.

In guinea pigs, following a single topical application of 5 µL of radiolabeled mustard gas to the cornea, radioactivity at 30 minutes after application, as expressed per unit weight, was greatest in the kidney followed by liver, lung, adipose tissue, adrenals, plasma, and muscle (Klain et al. 1991). At 2 and 5 hours postadministration, the greatest radioactivity per unit weight was again measured in the kidney, whereas the level in the plasma increased and that in the liver and lung decreased with postadministration time. Expressed per organ, the liver contained the highest level of radioactivity, followed by the kidney and lung. At 30 minutes postapplication, radioactivity was widely distributed in the guinea pig eye; the choroid/sclera portion contained the highest level followed by cornea, retina, and lens. Low levels were also detected in the aqueous and vitreous humors. At 5 hours, the only eye compartment in which the radioactivity level had decreased significantly from the 30 minutes value was the choroid/sclera portion.

3.4.2.4 Other Routes of Exposure

Boursnell et al. (1946) observed significant radioactivity levels in the kidney, lung, and liver of rabbits after intravenous injection of 5 mg/kg of radiolabeled mustard gas. Lower levels of radioactivity were also detected in bone marrow, spleen, stomach wall, duodenal wall, brain, heart, muscle, skin, and thyroid. Six hours after intravenous injection of 8.2 mg/kg of radiolabeled mustard gas into male hairless guinea pigs, radiolabel was distributed in decreasing concentrations to the bone marrow, liver, spleen, blood, and lung (Langenberg et al. 1998). In the rat, mustard gas is quickly and widely distributed (Maisonneuve et al. 1993, 1994; Zhang and Wu 1987). Maisonneuve et al. (1993) reported a distribution

volume of 74.4 L/kg and a half-life of 5.6 minutes following intravenous bolus administration of 10 mg/kg (3 LD_{50}) of mustard gas in the rat. The concentration of unchanged mustard gas in the blood decreased quickly in the first half hour, but low levels were detectable up to 8 hours after administration. The large volume of distribution, greater than the volume of body water, suggests a wide distribution of mustard gas throughout the animal. A quantitative distribution analysis was performed by Maisonneuve et al. (1994) in rats intravenously injected with radiolabeled mustard gas. Radioactivity was detected in blood, plasma, kidney, liver, intestine and stomach, heart, lung, brain, spleen, eyes, testicle, and adrenal gland. From 10 minutes to 6 hours after administration, the liver and kidney had higher radiolabel concentrations than the blood. The organs with the lowest levels of radioactivity were the brain, spleen, eye, and testicle. Maximum radioactivity levels in the organs were reached between 2 and 3 hours after injection. Total radioactivity in any organ did not exceed 4% of the administered dose. Most of the administered radioactivity was recovered in the muscle; 51% measured in muscle at 5 minutes, 36% in muscle at 3 hours, 3% in fat at 35 minutes, 10% in skin at 35 minutes (radioactivity peaked in fat and skin at 35 minutes).

In vitro studies of plasma and red blood cells treated with radiolabeled mustard gas indicate a high affinity of mustard gas toward red blood cells (Maisonneuve et al. 1993). The mean equilibrium red blood cell/plasma radiolabel concentration ratios for treatments with 4 and 400 µg/mL radiolabeled mustard gas were 2.12 and 4.15, respectively.

Radiolabeled mustard gas administered in rats to the femoral or jugular veins resulted in different organ distribution patterns. Subsequent to femoral vein injection, the injected leg was a site of significant mustard gas distribution, whereas jugular vein injection did not result in significant accumulation in the lung (Maisonneuve et al. 1994). The heart, lung, brain, and spleen received greater proportionate shares of radioactivity 35 minutes after jugular vein injection compared to femoral vein administration.

In the eyes of rats examined 4 hours after subcutaneous injection in the dorsal area with 10 µL of undiluted radiolabeled mustard gas, the largest amount of radioactivity was found in the pooled aqueous and vitreous humors (70%), followed by retina (12%), choroid/sclera (8%), lens (6%), and cornea (3%) (Klain et al. 1991).

3.4.3 Metabolism

The metabolism of mustard gas has not been studied extensively. Metabolic pathways including direct alkylation reactions, glutathione reactions, hydrolysis, and oxidation are presumed based on the finding of mustard gas DNA adducts in tissues and the identification of urinary products.

3.4.3.1 Inhalation Exposure

Mustard gas DNA adducts were found in the nasal epithelium, nasopharynx, larynx, carina, lung, spleen, and bone marrow of hairless guinea pigs after nose-only exposure to mustard gas (Langenberg et al. 1998).

3.4.3.2 Oral Exposure

No studies were located regarding metabolism in humans or animals after oral exposure to mustard gas.

3.4.3.3 Dermal Exposure

Studies of casualties of the Iran-Iraq War have identified significant amounts of mustard gas metabolite, thiodyglycol, in human urine more than a week after mustard gas exposure (Wils et al. 1985, 1988). The presence of this urinary biotransformation product is consistent with findings in animal studies discussed below in which mustard gas was administered by alternate routes. Sandelowsky et al. (1992) reported the detection of mustard gas metabolite, 4-met-1-imid-thiodiglycol, in plasma and urine following dermal exposure of mustard gas in pigs.

3.4.3.4 Other Routes of Exposure

Rats and mice were injected intraperitoneally with radiolabeled mustard gas, bis-2-chloroethyl-sulfide-^{35}S (Davison et al. 1961). The metabolism of this substance is apparently largely due to glutathione reactions, hydrolysis, and oxidation, since the major urinary metabolites were glutathione-bis-2-chloroethyl sulfide conjugates (45% of total), thiodiglycol and conjugates (14%), and sulfone products (20%). Slightly different results were reported by Roberts and Warwick (1963), who found that at least 50% of the urinary metabolites in rats was a conjugated form of bis-cysteinyl-ethylsulphone.

Thiodiglycol accounted for 15–20% of the urinary radioactivity, and 10–15% was a sulfide. Black et al. (1992b) investigated the metabolism of mustard gas similarly in the rat. Many metabolites were present in the urine, nine of which were identified as thiodglycol sulphoxide, 1,1'-sulphonybis[2-(methyl-sulphinyl)ethane], 1-[S-(N-acetylcysteinyl)]-2-(ethenylsulphonyl)ethane, 1-methylsulphinyl-2-[2-(methylthio)ethylsulphonyl]ethane, two diastereoisomers of 1-[S-(N-acetylcysteinyl)]-2-(2-chloroethyl-sulphinyl)ethane, 1,1'-sulphinybis[2-chloroethane], 1,1'-sulphonybis[2-S(N-acetylcysteinyl)ethane], and 1-[S-(N-acetylcysteinyl)]-2-(2-chloroethylsulphonyl)ethane, allowing the construction of a putative metabolic pathway (Black et al. 1992b). Black et al. (1992b), while confirming the major metabolic transformations of Davison et al. (1961), identified thiodiglycol sulphoxide as the major urinary excretion product and not the initial hydrolysis product thiodiglycol. The finding of metabolites 1,1'-sulphonybis-[2-(methylsulphinyl)ethane] and 1-methylsulphinyl-2-[2-(methylthio)ethylsulphonyl]ethane revealed a pathway for the degradation of glutathione conjugates formed via the action of enzyme β-lyase on cysteine conjugates. Renal β-lyase metabolism has also been implicated in the formation of nephrotoxic intermediates from halogenated alkenes.

A comparison of unchanged radiolabeled mustard gas and total radiolabel concentrations in the blood following intravenous bolus administration of radiolabeled mustard gas in rats indicated that much of the mustard gas is metabolized with a half-hour after administration (Maisonneuve et al. 1993).

3.4.4 Elimination and Excretion

Urinary excretion is the primary route of elimination for mustard gas and/or its metabolites.

3.4.4.1 Inhalation Exposure

People who were exposed to mustard gas during the Iran-Iraq War could have absorbed the material through the lungs or through the skin. One of the breakdown products of mustard gas, thiodiglycol, has been detected in the urine of these people (Wils et al. 1985). These authors also report that thiodiglycol is found in unexposed persons and cannot be used to determine the exact level of mustard gas exposure, although it could possibly be used to show large exposures. Unmetabolized mustard gas was also found in urine and feces samples from two Iran-Iraq War victims (Heyndrickx and Heyndrickx 1984; Mandl and Freilinger 1984; Pauser et al. 1984; Vycudilik 1985). No studies regarding animal excretion data from inhalation exposure are available.

3.4.4.2 Oral Exposure

No studies were located regarding excretion in humans or animals after oral exposure to mustard gas.

3.4.4.3 Dermal Exposure

People who were exposed to mustard gas during the Iran-Iraq War could have absorbed the material through the lungs or through the skin. One of the breakdown products of mustard gas, thiodiglycol, has been detected in the urine of these people (Wils et al. 1985). These authors also report that thiodiglycol is found in unexposed persons, and cannot be used to determine level of mustard gas exposure.

Jakubowski et al. (2000) measured the excretion of thiodiglycol in human urine following an accidental mustard gas exposure. In contrast to Wils et al. (1985), detectable levels of thiodiglycol in urine were measured for 13 days after exposure. The patient's urine was random sampled for 6 months after exposure and no further thiodiglycol elimination was detected. Maximum thiodiglycol excretion was seen on postexposure day 4. First-order elimination kinetics were observed and the half-life of thiodiglycol elimination was estimated to be 1.2 days.

Hambrook et al. (1992) reported that in the rat, following a 6-hour cutaneous exposure to radiolabeled mustard gas with occlusion, the urinary excretion of radiolabel had a half-life of 1.4 days; the half-life of excretion in feces, which varied slightly with dose, was approximately 1.6 days. Most of the radioactivity was found in the urine. Most of the dose was eliminated by 3 days; however, urinary excretion of radiolabel continued for greater than 3 months.

3.4.4.4 Other Routes of Exposure

Two terminal cancer patients were injected intravenously with radiolabeled mustard gas dissolved in ethanol (Davison et al. 1961). Several minutes after administration, 80–90% of the radioactivity was cleared from the blood. The residual level remained constant in both plasma and cells for at least 2 days, suggesting binding to some blood constituent. Excretion of 21% of the radioactivity in the urine occurred within 3 days. The metabolites found in the liver were the same as those in rats, suggesting that human metabolism is similar to rat metabolism for this chemical.

The major route of elimination of radioactivity in the rat, after intravenous injection of radiolabeled mustard gas is by the kidney (Boursnell et al. 1946; Davison et al. 1961; Hambrook et al. 1992; Maisonneuve et al. 1993). Maisonneuve et al. (1993) reported a blood clearance of 21 L/hours-kg and elimination half-life of 3.59 hours from blood concentration data following intravenous bolus administration of 10 mg/kg (3 LD_{50}) of radiolabeled mustard gas in the rat. Similarly to that found in humans, a residual constant level of radioactivity was found in blood for 2 days after exposure; a slight increase in the residual level was observed between 2 and 4 days. The largest overall recovery of radioactivity was in urine, with about 65% of the administered dose excreted during 24 hours and 80% during 96 hours, a much higher percentage than that reported for humans (Davison et al. 1961). Fecal excretion accounted for <3% of the administered dose during 96 hours (Maisonneuve et al. 1993).

Rats and mice that were injected intraperitoneally with radiolabeled mustard gas excreted 50–78% of the radioactivity within 1 day and 90% within 3–5 days in the urine (Black et al. 1992a; Davison et al. 1961; Roberts and Warwick 1963; Smith et al. 1958). Twelve hours after intraperitoneal injection, 6% was excreted in the feces and 0.05% in the expired air (Davison et al. 1961).

Hambrook et al. (1992) measured the excretion of radiolabel in urine and feces in the rat following intravenous or intraperitoneal injection of radiolabeled mustard gas. The half-life varied little with dose, route, or excretion type and an average value of 1.4 days was reported. The pattern of excretion was similar after intraperitoneal and intravenous injections. Most of the dose was eliminated by 3 days; however, urinary excretion of radiolabel continued for greater than 3 months. About 65% of absorbed radiolabel was found in the urine and 11% in feces within 24 hours after administration.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

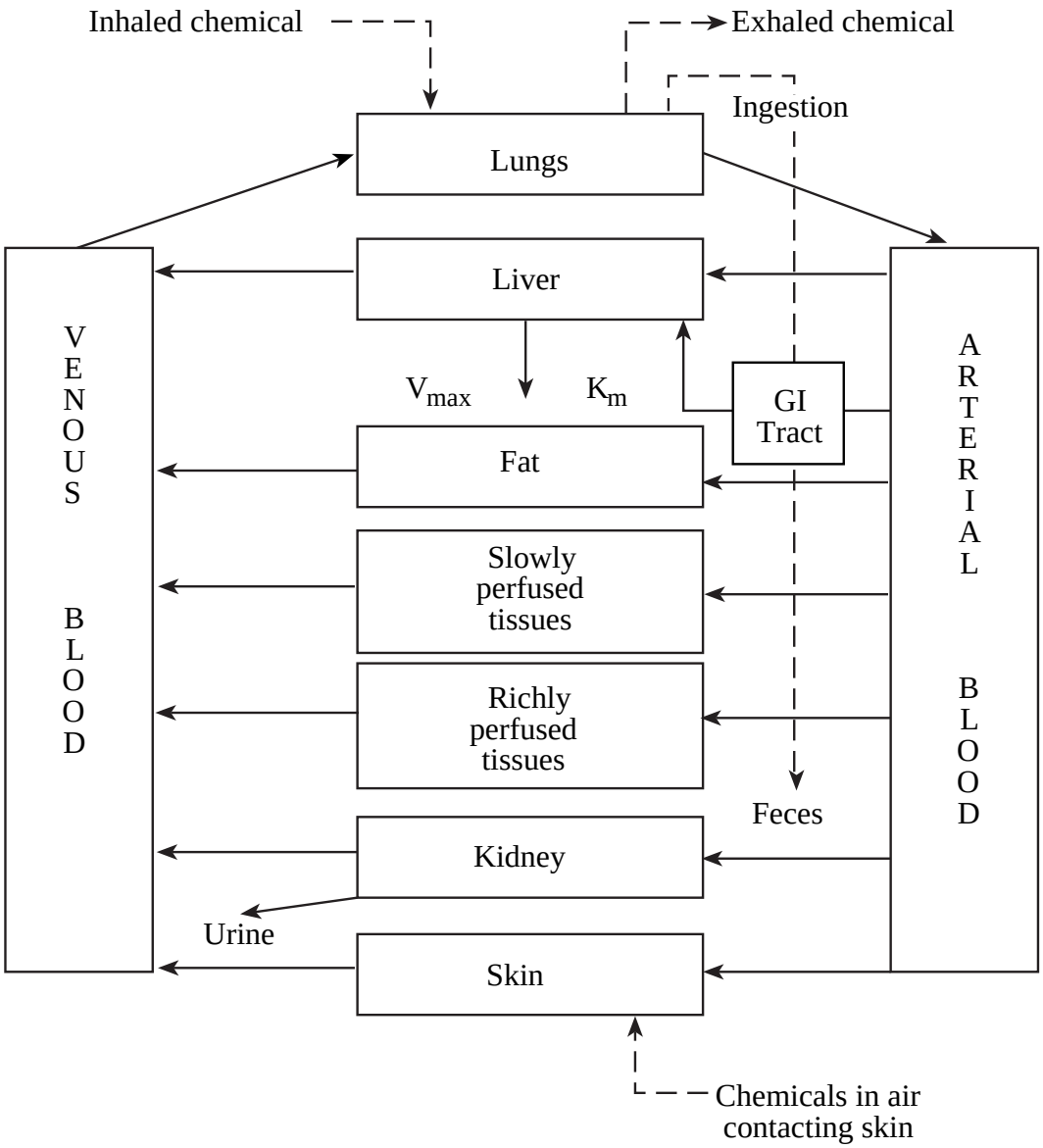
PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-3 shows a conceptualized representation of a PBPK model.

Figure 3-3. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

Source: adapted from Krishnan et al. 1994

No PBPK models exist for mustard gas. Toxicokinetic information is insufficient for modeling.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. Mustard gas is slightly soluble in water, but both the liquid and vapor forms are readily soluble in oils, fats, alcohol, and organic solvents. Because of its high lipid solubility, mustard gas quickly penetrates the lipid cell membrane.

Distribution. It has been estimated that about 12% of dermally absorbed mustard gas reacts with components in skin and the remainder is distributed in greatest proportion to the kidney and fairly evenly throughout the rest of the body as unreacted mustard gas or hydrolyzed mustard gas. In studies with radiolabeled mustard gas, tissue radioactivity levels increased as early as 5 minutes after intravenous injection and 15 minutes after percutaneous administration.

Metabolism. Mustard gas is presumed to be biotransformed by direct alkylation reactions, glutathione reactions, hydrolysis, and oxidation are based on the finding of mustard gas DNA adducts in tissues and the identification of urinary products.

Excretion. Urinary excretion is the primary route of elimination for mustard gas and/or its metabolites. In humans, elimination follows first-order kinetics and the half-life of thiodiglycol elimination is estimated to be 1.2 days (Jakubowski et al. 2000).

3.5.2 Mechanisms of Toxicity

At the cellular level, mustard gas interacts with nucleophiles on the cell membrane, at intracellular sites, and with nucleic acids (Papirmeister et al. 1991). While mustard gas is able to alkylate DNA, RNA, and proteins affecting a variety of cell functions, including altering proteins that have been coded by alkylated RNA and structurally altering cell membranes, DNA is the most functionally sensitive target of mustard gas in cells. Low doses of mustard gas can inhibit cell division due to its ability to cross-link complementary strands of DNA (Papirmeister 1993). Transcription, translation, and enzyme catalysis, cellular activities that are dependent on biological entities of much lower molecular size than

chromosomal DNA, are much less sensitive to mustard gas. Thus, cells that are prevented from synthesizing DNA continue to generate energy and synthesize RNA and proteins. As a result of this unbalanced metabolism, cells may enlarge, differentiate, or be induced to synthesize high levels of certain proteins. While some of these proteins may protect cells, others may hasten cell death.

Mechanisms of the toxicity of mustard gas have been postulated, but none have been demonstrated with certainty (Papirmeister 1993, 1994; Somani and Babu 1989; Whitfield 1987). As discussed in Section 3.3, it appears that different mechanisms are responsible for the acute and delayed effects of mustard gas and that additional mechanisms besides genotoxicity mechanisms are responsible for mustard gas vesication since acute skin injury develops at a time much earlier than expected from genotoxic effects. Also, tissue injury does not develop when low, therapeutically effective doses of mustard gas are used to control the hyperproliferation of psoratic keratinocytes. While the mechanisms of mustard gas toxicity are not currently fully understood, one hypothesis for mustard gas cytotoxicity involves poly(adenosine diphosphoribose) polymerase (PADPRP). The following mechanism for skin damage has been proposed: mustard gas alkylates DNA, which causes DNA breaks; numerous mustard gas-induced DNA strand breaks cause activation of nuclear repair enzyme PADPRP. This causes cellular depletion of nicotinamide adenine dinucleotide (NAD^+), which decreases glycolysis, which leads to protease release and cellular injury. Dermal-epidermal separation and blister formation may involve the fragmentation of anchoring filaments by protease released from moribund or dead cells (Papirmeister 1993). Clark and Smith (1993) showed that mustard gas treatment of HeLa cells produces a rapid stimulation of PADPRP activity, followed 2 hours later by a decline in NAD^+ levels. Several other studies provide partial support for the hypothesis, but hint that additional pathways may be involved. The hypothesis is almost fully validated in a study by Meier and Kelly (1993), in which PADPRP inhibitors prevent the mustard gas-induced losses of ATP, NAD^+, and viability in human peripheral blood lymphocytes. However, their observation that ATP levels decline before NAD^+ deviates from the expected response. Niacinamide, an inhibitor of PADPRP and a substrate for NAD synthesis reduced mustard gas-induced loss in NAD (Meier et al. 1987; Mol et al. 1989, 1991; Papirmeister et al. 1985; Smith et al. 1990) and ATP (Meier et al. 1996). 3-Aminobenzamide, an inhibitor of PADPRP but not a substrate for NAD synthesis, also reduced mustard gas-induced loss in ATP (Meier et al. 1996). Niacin, a substrate for NAD synthesis, which does not effect PADPRP, failed to prevent mustard gas-induced loss of ATP (Meier et al. 1996). These findings support the hypothesis that PADPRP plays a substantial role in mustard gas-initiated biochemical changes. Cowan et al. (1993) observed that although niacinamide-attenuated mustard gas-induced increases in protease activity *in vitro* and *in vivo*, it did not

eliminate them, suggesting that pathways other than the one involving PADPRP may contribute to the increase in protease activity. Yourick et al. (1991, 1993) noted that while pretreatment with niacinamide reduced the incidence of mustard gas-induced microvesiculation in hairless guinea pig skin, the prediction of the PADPRP hypothesis, that the loss of NAD^+ precedes tissue injury, was not upheld. Martens and Smith (1993) demonstrated that whereas mustard gas treatment of human epidermal keratinocytes (HEK) produces a dose-related depletion of NAD^+ and inhibition of glucose metabolism, preceding cell death, niacinamide did not prevent the inhibition of glycolysis, suggesting that in HEK, other energy-depleting mechanisms may be involved in mustard gas cytotoxicity. In contradiction to the hypothesis, results in rat keratinocytes exposed to mustard gas indicate that depletion of NAD is not a prerequisite for cell death (Lin et al. 1994). At doses lower than 50 µM, DNA content, viable cell number, and the proliferative capacity of the culture, as assessed by thymidine incorporation, were all reduced, whereas the total NAD level (NAD^+ plus NADH) was not changed. Also supplementing the culture with nicotinamide after exposure to mustard gas did not reverse the decrease in DNA content.

As another hypothesis for mustard gas-induced cytotoxicity, Whitfield (1987) suggested that mustard gas alkylation of glutathione (GSH) removes one of the major cellular defense mechanisms against electrophilic compounds and oxidants. Once GSH is depleted, electrophiles such as mustard gas or endogenously-generated reactive oxygen species eventually inactivate critical sulfhydryl proteins involved in calcium homeostasis and/or modify cytoskeletal elements. The subsequent inability of cells to maintain the low intracellular calcium concentration causes activation of catabolic processes leading to cell damage and death. In partial support of this hypothesis, Ray et al. (1993) demonstrated that treatment of neuroblastoma cells and HEKs with mustard gas causes depletion of GSH, raises the level of intracellular calcium, and stimulates phospholipase A_2, processes that precede and ultimately lead to membrane damage and cell death. Also, increasing cellular GSH levels decreased the toxic effects of mustard gas in human peripheral blood lymphocytes (Gross and Smith 1993).

Apoptosis may be a mechanism by which mustard gas exerts its cytotoxic effects. In keratinocytes incubated with mustard gas, p53 (a promoter of apoptosis) levels increases, while levels of bcl-2 (a suppressor of apoptosis) decreased (Rosenthal et al. 1998). The immunostaining pattern of these two markers in mustard gas treated skin excised from weanling pigs also suggests the involvement of apoptosis in cell death secondary to mustard gas exposure (Smith et al. 1997a). Thymocytes, isolated from rats, and incubated with mustard gas showed an increase in the production of DNA fragments

characteristic of apoptosis (Michaelson 2000). It is possible that mustard gas toxicity involves several independent or interacting pathways, some aspects of the various hypotheses.

Cell cycle kinetics are involved in the cytotoxic processes following mustard gas exposure. Mustard gas-induced damage at subvesicating concentrations (<50 μM) to genomic DNA in cultured HEK resulted in a dose-related reversible block at the G_2/M phase of the cell cycle (Smith et al. 1993). Okadaic acid and calyculin A, inhibitors of protein phosphatase 2A (PP2A), completely reversed the mustard gas-induced G_2/M block, whereas tautomycin, an inhibitor of protein phosphatase 1, was ineffective at reversing the block (Hart and Schlager 1997). As total cellular PP2A was not affected by mustard gas treatment; these results suggest that PP2A is involved in the G_2/M block produced by exposure of HEK to low concentrations of mustard gas. Exposure of human peripheral blood lymphocytes (PBL) to vesicating equivalent concentrations of mustard gas ($50 μM) resulted in irreversible blockage at the G1/S interface (Smith et al. 1998). DNA became terminally fragmented.

Theories have been proposed that blistering induced by mustard gas may involve cytokine production and a secondary inflammatory response (Dannenberg and Tsuruta 1993; Graham et al. 1993; Papirmeister et al. 1991). In the trachea as in the skin, mustard gas appears to preferentially damage the cells that are the most active in regeneration after aggression, basal cells located above the dermal papillae in skin (Papirmeister et al. 1991) and epithelial secretory cells in the trachea (Calvet et al. 1996). In the cell, DNA and proteins are the main targets for mustard gas alkylation; therefore, it is not unexpected that the most severe lesions effect cells with the greatest progenitorial and metabolic capacity. Eosinophils, known to produce growth factor and cytokines, were reduced in guinea pigs at 2 weeks postexposure, which may influence epithelial regeneration and result in the characteristic slow lesion repair or recovery (Calvet et al. 1996). The literature contains conflicting reports of mustard gas effects on cytokines. In cultured HEK treated with 1–100 μM mustard gas, Pu et al. (1995) observed a dose-related increase in IL-1α at 72 hours after exposure. Zhang et al. (1995) also measured an increase in IL-1α in isolated perfused porcine skin treated with mustard gas at 5 hours after exposure. In contrast, Kurt et al. (1998) who tested the effects of mustard gas on both adult and neonatal HEK, reported a dose-related decrease in IL-1α in cultured adult HEK treated with 0.5 and 1.0 mM mustard gas; however, only a minimal change in IL-1α was seen in neonatal HEK. Mustard gas applied to the mouse ear resulted in an increase in IL-6 levels at 6 and 18 hours postexposure, whereas IL-1β and TNF-α levels were unchanged (Casillas et al. 1996). Kurt et al. (1998) reported that in both neonatal and adult HEK, TNF-α was increased at 0.5 mM and decreased at 1.0 mM mustard gas, whereas IL-1β, IL-6, and IL-8 were increased at both

concentrations. While IL-1α and IL-1β share the same biological activity and recognize the same receptors on target cells, Kurt et al. (1998) suggest that the differences in the amount of each cytokine released relative to the distribution in HEK support different mechanisms of action for mustard gas with IL-1α and IL-1β. Since the decrease in IL-1β was the only cytokine of those studied with significant decreases in both neonatal and adult cell types and at both concentrations, Kurt et al. (1998) hypothesized a direct effect of mustard gas on IL-1β and indirect actions on the other cytokines.

In order to investigation possible mechanisms of blistering, urokinase, one of two mammalian activators for converting plasminogen into active plasmin, was investigated *in vitro* in cultured 3T3 fibroblasts exposed to 100 μM mustard gas (Detheux et al. 1997). Plasmin is a wide-spectrum serine protease, which is capable of degrading most extracellular and basement membrane proteins. Twenty-four hours after exposure, urokinase activity was increased 20-fold compared to control cells. The significance of this proteolytic response in the pathogenesis of blistering is not yet understood.

There have been several studies of protein alkylation by mustard gas with possible relevance to blister formation. A potential target for mustard gas alykation is uncein, an anchoring filament-associated antigen thought to play a role in maintaining the integrity of the dermal-epidermal basement membrane zone. Fractionation by SDS-PAGE and immonofluorescent staining of uncein treated with mustard gas indicated that mustard gas chemically modified uncein (Zhang et al. 1998). Male Yorkshire cross weanling pigs were exposed dermally to two vesicating doses, estimated at 21,000 and 42,000 mg-minute/m^3, of mustard gas (Smith et al. 1997a). Immunostaining of excised treated skin revealed a progressive decrease with eventual loss of expression of GB3, an antibody to basement membrane protein, laminin 5, during the time of vesiculation at both doses. Desmosomal proteins, cellular fibronectin, laminin 1, collagen IV, and collagen VII showed no change or inconsistent changes during the same period. The laminins are cystein-rich proteins with multiple thiol groups available for alkylation by mustard gas. The pattern of immunostaining for laminin 5 was consistent with electron microscopy findings showing fragmentation of anchoring filaments at the time of vesication and suggests that disruption of laminin 5 may be a factor in mustard gas-induced blistering. Laminin 5 regeneration occurs early after injury, whereas cutaneous lesions are slow-healing with no evidence of re-epithelialization at 7 days after exposure in a hairless guinea pig model. The authors suggest that residual alkylated laminin 5 and laminin 1 fragments could inhibit the functioning of the newly formed laminin 5.

DNA arrays were used to study the differential gene expression changes that occur within human epidermal keratinocytes after exposure to mustard gas (Platteborze 2000). Several genes were identified that exhibited significant transcriptional upregulation that could have roles in early mustard gas injury. Transmembrane serine protease hepsin, which is thought to be involved in cell growth, differentiation, and maintenance of morphology, was upregulated about 8-fold at 10–30 minutes after exposure. Heparin sulfate proteoglycan 2 (HSPG2) was upregulated about 13-fold at 10 minutes and about 8-fold at 30 minutes after exposure. HSPG2 is an integral component of basement membranes and is proposed to be involved in cell binding, basement membrane assembly, calcium binding, LDL metabolism, activation of serine protease inhibitors, and the anchorage of acetylcholinesterase (AChE) to the extracellular matrix of the neuromuscular junction. In addition, heparin sulfate chains carry a fixed negative charge, which is thought to participate in the selective permeability of basement membranes. Human periodic tryptophan protein 2 (yeast) homolog (PWP2H) was also significantly overexpressed, about 7-fold at 10 minutes and about 14-fold at 30 minutes. At present, little is known about the function of PWP2H. A notable absence of upregulation of nucleotide repair genes, ERCC1 (Excision Repair Cross-Complementing repair deficiency group 1) and ERCC2, and enzyme poly(ADP-ribose) polymerase (PADPRP) at 10 and 30 minutes postexposure suggests that the recognition or response of human epidermal keratinocytes to mustard gas genotoxicity is delayed, since poly(ADP-ribose) polymerase (PADPRP) activation was observed at 4 hours after exposure.

A dose-dependent inhibitory effect of mustard gas on the heat shock response was found in mononuclear human cells (Sterri 1993). The effect was fully developed at subvesicating doses and was strongly dependent on the order of the exposures to mustard gas and stress effector. Heat shock protein expression was inhibited in cells exposed to mustard gas and subsequently heat shocked, whereas cells that were heat shocked first and then exposed to mustard gas continued with the normal heat shock response. These results point to both transcriptional and translational sites of effect. The mechanistic coupling between the stress response and mustard gas remains to be understood.

Sawyer et al. (1996) examined the possibility that the toxicity of mustard gas is due to the induction or activation of nitric oxide synthase (NOS). L-nitroarginine methyl ester (L-NAME), an arginine analog inhibitor of NOS, was found to confer protection to mature primary cultures of chick embryo forebrain neurons against the toxicity of mustard gas when administered as a pretreatment or up to 3 hours postexposure. No protection was evident in immature (1-day-old) cultures. While NOS requires L-arginine as a substrate, mustard gas toxicity and L-NAME protection were independent of L-arginine

concentration. In contrast to L-NAME, L-thiocitrulline (L-TC), another arginine analog NOS inhibitor, was found to protect immature cultures of neurons against mustard gas, as well as mature cultures (Sawyer et al. 1998). L-TC increased the LC_{50} of mustard gas by approximately 800 and 1,500% with 1-hour and 24-hour pretreatments, respectively. The protection conferred by L-TC was persistent, unlike L-NAME whose protection was dependent on its continued presence, suggesting that these closely related arginine analogs act at different sites to exert their effects (Sawyer et al. 1996, 1998). A synergistic protective effect was found in mature neuron cultures pretreated with both L-NAME and L-TC (Sawyer 1998). Whereas 1-hour pretreatment with L-NAME and L-TC increased the LC_{50} of mustard gas by approximately 200 and 800%, respectively, together up to 1,500% protection was conferred in mature cultures. Based on these findings, Sawyer (1998) proposed that mustard gas initiates its toxicity rapidly through a cell-surface mediated event, that can be blocked by L-TC, followed by signal transduction into the cell with an additional event manifested several hours later. The role of NOS in mustard gas toxicity remains unclear; however, these arginine analog NOS inhibitors provide protective effects, apparently not mediated through inhibition of NOS.

A study by Zhang et al. (1995) of the protective effects of four pharmacological agents in mustard gas-treated isolated perfuse porcine skin flap (IPPSF) suggests that different mechanisms are involved in the production of mustard gas-induced dark basal cells, microvesicles, and vascular response. Reduction of mustard gas-induced dark basal cells was observed with mustard gas scavengers, sodium thiosulfate and cysteine, with niacinamide, an inhibitor of poly(adenosine diphosphoribose) polymerase (PADPRP) and a substrate for NAD synthesis, and with cycooxygenase inhibitor indomethacin. Treatments with niacinamide and indomethacin, but not sodium thiosulfate or cysteine, resulted in an inhibition of the vascular response in IPPSF exposed to mustard gas. Of the four agents, microvesicles were only partially prevented in the indomethacin-perfused IPPSF.

The toxic effects of sulfur mustard have been attributed to DNA modification with the formation of 7-hydroxyethylthioethyl guanine, 3-hydroxyethylthioethyl adenine and the cross-link, di-(2-guanin-7-yl-ethyl)sulfide. Bacterial 3-methyladenine DNA glycosylase II (Gly II) was found to releases both 3-hydroxyethylthioethyl adenine and 7-hydroxyethylthioethyl guanine from calf thymus DNA was modified with [14C]sulfur mustard, suggesting that glycosylase action may play a role in protecting cells from the toxic effects of mustard gas (Matijasevic et al. 1996).

Mustard gas was found to inhibit blood cell and tissue antioxidant enzyme activities in rats following topical application, which could impair cytoprtective defense mechanisms (Husain et al. 1996). Enzyme activities were measured at 24 hours after dermal treatment with 98 mg/mg (0.5 LD_{50}) of mustard gas. Superoxide dismutase (SOD) activity decreased significantly, 70% in white blood cells, 65% in platelets, 72% in the spleen, and 29% in brain. SOD activity in red blood cells, liver, and kidney did not change significantly following treatment. Catalase activity decreased significantly 54% in white blood cells, 23% in red blood cells and 51% in spleen, whereas activity levels in platelets, liver, kidney, and brain were not significantly altered. Glutathione peroxidase (GSH-Px) activity, as a consequence of glutathione and NADPH depletion, decreased significantly in white blood cells (42%), spleen (43%), and liver (22%). Glutathione activities in red blood cells, platelets, kidney, and brain were within 10% of control values.

A significant depletion of GSH of blood and liver was also observed in mice following dermal application of 38.7 or 77.4 mg/kg of mustard gas (Vijayaraghavan et al. 1991).

3.5.3 Animal-to-Human Extrapolations

Various models consisting of human peripheral blood lymphocytes, human skin grafts, porcine skin flaps in explant culture, human epidermal keratinocytes in culture, human eyes, hairless guinea pigs, and stratified rat epidermal cultures have been developed to study the biochemical events in sulfur mustard toxicity. As discussed in Section 3.2.1.2, short-term respiratory effects similar to those described in humans have been reported in experimental animals, which suggests that knowledge obtained regarding respiratory effects in animal models can be usefully applied to humans.

Unless people drink mustard gas directly from the container, they would not be exposed to it acutely via the oral route because it hydrolyzes quickly in water, so more data in this area is not needed. Laboratory animals with fur do not have sweat glands on most of their body and do not provide optimal models for dermal exposure.

3.6 Toxicities Mediated Through the Neuroendocrine Axis

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals

with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Colborn and Thomas (1992) and again by Colborn (1993), was also used in 1996 when Congress mandated the Environmental Protection Agency (EPA) to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), which in 1998 completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Hoel et al. 1992; Giwercman et al. 1993; Berger 1994).

It is possible that mustard gas modifies the feedback of endogenous hormones and, through the complex interactions of central nervous system and endocrine function regulation, behavior (i.e., libido). In a survey of 800 Iranian men who were exposed to mustard gas during the Iran-Iraq War, 279 men (34.8%) reported decreased libido, 342 (42.8%) reported no change, 6 (0.8%) reported increased libido, and 173 (21.6%) did not respond to this survey question (Pour-Jafari and Moushtaghi 1992). Of these men, 86.6% still suffered symptoms from chemical injury, namely lung and skin lesions.

There is limited evidence to suggest that mustard gas affects FSH levels and thus plays a role in reproductive function. The time course of changes in serum concentrations of total and free testosterone, LH, DS, FSH, 17 α-OH progesterone, and prolactin were studied in 16 men during the first 3 months after chemically confirmed exposure to chemical weapons containing mustard gas in 1987 during the Iran-Iraq War (Azizi et al. 1995). A group of 34 healthy unexposed men of similar age served as controls. Released from the pituitary, LH stimulates the Leydig cells to produce testosterone, while FSH stimulates the Sertoli cells to produce sperm. At 1 week after exposure, total testosterone, free testosterone, and DS were significantly lower, 57, 72, and 53%, respectively, in exposed men than in controls, while levels of the remaining hormones were comparable between groups. Total testosterone, free testosterone, and DS levels continued to decrease during the first 5 weeks after exposure. At 1 week, 4 of 16 exposed men (25%) had serum testosterone levels that were reduced by >60% below the control average; by the 5th week, the number increased to 11 (69%). DS mean values reached as low as 18% of the mean of control subjects. After the 5th week, these three hormone levels increased returning to normal levels at 12 weeks after injury. Small but significant increases in mean serum concentration of LH at the 3rd week and that of FSH and prolactin at the 5th week were measured. Normal levels of LH, FSH, and prolactin were measured at 12 weeks. FSH and LH response levels to 100 µg of gonadotropin releasing hormone (GnRH) administered intravenously during the first week after exposure, were subnormal in four of five patients. While testosterone levels in these men returned to normal 12 weeks after exposure, in a survey of 800 Iranian men who were exposed to mustard gas during the Iran-Iraq War, 279 men (34.8%) reported decreased libido, 342 (42.8%) reported no change, 6 (0.8%) reported increased libido, and 173 (21.6%) did not respond to this survey question (Pour-Jafari and Moushtaghi 1992). Of these men, 86.6% still suffered symptoms from chemical injury, namely lung and skin lesions.

In a follow-up study of 42 men, ages 18–37, injured by mustard gas during the Iran-Iraq War, serum testosterone, LH, and prolactin concentrations were normal in all men 1–3 years following exposure (Azizi et al. 1995). A comparison of the mean serum FSH concentration in 13 subjects with sperm count below 20 million and in 20 subjects with sperm counts above 60 million, revealed a nearly 2-fold increase in FSH concentration in the those with the lower sperm count; the increased FSH level was 38% above the mean FSH concentration in a group of 34 health unexposed males. Inhibition of spermatogenesis was also observed in male mice following intravenous injection of mustard gas (Graef et al. 1948). Elevated FSH has been correlated clinically with testicular failure, germinal aplasia, or hypergonadotropic hypogonadism. It appears unlikely that alteration of FSH levels is related to the

effect of mustard gas on the pituitary since LH levels were unaffected in males. A possible target is inhibin secretion by testes Sertoli cells, which suppresses pituitary FSH secretion.

Administration of mustard gas did not affect the reproductive potential of female mice because the fertility of the mice was not altered and no injurious effects were observed in the ovaries (Graef et al. 1948). Chronic (52 weeks) inhalation exposure of male rats to mustard gas (0.1 mg/m^3) was reported to produce significant dominant lethal mutation rates (a maximum of 9.4% at 12–52 weeks), but exposure of pregnant females to the same concentration for a shorter time interval did not (Rozmiarek et al. 1973).

McNamara et al. (1975) subsequently concluded from these same data that there were no differences between the control and experimental groups and no evidence of mutagenesis. The conflict between these two reports is not readily resolvable, but the fetal mortality values presented by McNamara et al. (1975) suggest at least a trend for dominant lethal effect. Complete control data and statistical analyses of the results are not presented, but percentages of fetal death at week 12 were 4.12, 4.24, and 21.05 for controls, 0.001, and 0.1 mg/m^3 exposure groups, respectively. In a dominant lethal study of mustard gas, rats were orally gavaged with 0.08, 0.2, or 0.5 mg/kg/day mustard gas 5 days/week for 10 weeks (Sasser et al. 1993). In female dominant lethality experiments, reproductive performance indicators (number of live or dead implants, resorptions, and preimplantation losses) in treated female rats mated to treated or nontreated males were not significantly different from controls. In male dominant lethality experiments (treated males were mated with untreated females), resorptions and preimplantation losses in the mid- and high-dose groups were significantly elevated. High-dose male sperm morphology data at all postexposure sampling times, 0, 5, and 12 weeks, showed a statistically significant decrease in the percentage of normal sperm. Blunthook and banana-shaped sperm heads were observed at 0, 5, and 12 weeks, whereas amorphous and short head abnormalities were observed only at 5 and 12 weeks. Overall, there was a total 2-fold increase in abnormal sperm heads in high-dose mustard gas-treated males. In summary, female fertility was not affected by these mustard gas exposures; however, a male dominant lethal effect was demonstrated at the mid and high doses of mustard gas. This lack of reproductive effect in female animals further supports the testes, rather than the pituitary, as the target organ in connection with possible mustard gas-induced alteration in FSH levels.

The time course of changes in thyroid indices, serum T3, T4, TSH, reverse T3, thyroglobulin and cortisol, plasma ACTH, and free T3 and T4 indexes (FT3I, FT4I) were studied in 13 male soldiers, ages 21–32 years, during the first 5 weeks after chemically confirmed exposure in 1987 during the Iran-Iraq

War to chemical weapons containing mustard gas (Azizi et al. 1993). A group of 34 healthy unexposed men of similar age served as controls. T4 and FT4I were not consistently affected following injury; compared to controls, significantly decreased values were measured at 1 and 5 weeks after exposure, and but values slightly above normal were measured at 3 weeks. T3 and FT3I were significantly lower (11–23%) than control at 1, 3, and 5 weeks after injury. Reverse T3 concentration in injured men was significantly higher (29%) than mean control value at 1 week, but was normal at weeks 3 and 5. TSH and thyroglobulin levels in the injured soldiers were comparable to controls during the 5 postexposure weeks. Cortisol was significantly higher (40%) than normal 1 week after exposure, within the normal range at week 3, and significantly decreased (50%) below normal at week 5. ACTH was significantly increased (57–80%) above the normal control value at 1, 3, and 5 weeks after exposure. No follow-up studies of thyroid indices were located to determine whether normal levels returned or if any chronic effects exist.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6 Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates

because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

Information on children's health effects is provided from reports of children exposed to mustard gas from air bombs during the Iran-Iraq War (Momeni and Aminjavaheri 1994). Clinical manifestations of mustard gas in the children included ocular, cutaneous, respiratory, gastrointestinal, hematological, and neurological effects similar to those seen in adults; however, the onset of symptoms in children was sooner than in adults and the severity was greater. Generally, irritant effects of mustard gas in adults are delayed by about 8 hours, whereas manifestations in children occurred as early as 4 hours after exposure. As in adults, the most severe effects were portal-of-entry effects to the eyes, skin, and respiratory tract as might be expected for a vesicant. Cough and vomiting were the first symptoms in children, but not in

adults. Genital manifestations were less frequent in children and teenagers (42%) than adults (70%); however, even within the group of children, the incidence and severity of genital lesions increased with age. Other skin lesions had no apparent age-relation. The only information regarding possible adverse developmental effects in humans is that provided by Pour-Jafari (1994b), which suggests an association between parental exposure to mustard gas and elevated rates for congenital malformations. Studies of animals exposed during pregnancy by oral gavage have indicated reduced fetal weight and reduced ossification of the vertebrae and/or sternebrae, but only at levels that were also toxic to the mother (DOA 1987b; Sasser et al. 1996a).

There is no information regarding pharmacokinetics of mustard gas in children nor it is known whether mustard gas can be stored and excreted in breast milk. There have been no direct measurements to determine whether mustard gas can cross the placenta. There is no information on whether mustard gas can be stored in maternal tissues and be mobilized during pregnancy or lactation. There is no information on the metabolism of mustard gas in children.

There are no biomarkers of exposure or effect for mustard gas that have been validated in children or in adults exposed as children. No studies were located regarding interactions of mustard gas with other chemicals in children or adults.

No information was located regarding pediatric-specific methods for reducing peak absorption following exposure to mustard gas, reducing body burden, or interfering with the mechanism of action for toxic effects. In addition, no data were located regarding whether methods for reducing toxic effects of mustard gas in adults might be contraindicated in children.

Kurt et al. (1998) report differential sensitivity related to cytokine release of cultured adult and neonatal human epidermal keratinocytes treated with mustard gas, but the significance of these findings are not known.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to mustard gas are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by mustard gas are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10 “Populations That Are Unusually Susceptible”.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Mustard Gas

It is possible that mustard gas itself may be detected in the urine if a person is exposed to very high levels. However, the need for low-level and retrospective detection of exposure has been illustrated in the attempts to clarify the causes of the significant number of postwar symptoms experienced by soldiers involved in the Persian Gulf War. Black et al. (1992a) identified, in addition to several other metabolites, thiodiglycol sulphoxide as the major urinary excretion product, and not the initial hydrolysis product thiodiglycol. In two subjects accidentally exposed to mustard gas, urine thiodiglycol sulphoxide concentrations were 20–35 times thiodyglycol concentrations (Black and Read 1995a). However, the use of thiodiglycol sulphoxide or thiodyglycol as biological markers for mustard gas poisoning is limited by their presence at low concentrations in normal human urine. Of the remaining metabolites, several are conjugates of mustard gas with N-acetylcysteine, most of which have poor mass spectrometric and/or gas chromatography properties mainly due to thermal instability (Black et al. 1991). Two closely related metabolites of mustard gas, 1,1'-sulphonylbis[2-(methylsulphinyl)ethane] and 1-methylsulphinyl-2-[2-methylthio)ethylsulphonyl]ethane, derived from the action of β-lyase on cysteine conjugates, have been detected in urine collected from Iran-Iraq War casualties of mustard gas poisoning (Black and Read 1995b). There were no background levels of these metabolites detected in human or rat urine (Black et al. 1991).

Since mustard gas is known to alkylate DNA, RNA, and proteins, initial attempts were made to detect mustard gas DNA adducts in urine, which may be released from dying cells (Somani and Babu 1989). N-alkylated purines, such as N7-hydroxyethylguanine, have been identified from enzymatic digests as active sites for mustard gas (Fidder et al. 1994; Niu et al. 1996; Somani and Babu 1989; Van der Schans et al. 1994). The N7-guanine adduct of mustard gas in DNA has been detected by immunochemical analysis in the blood of two victims of the Iran-Iraq War (Benschop et al. 1997). The complications that arise to isolate double-stranded DNA from biological samples and to make the DNA single-stranded without destruction of the mustard gas adducts result in about a 20-fold higher limit for adduct detection in DNA from human blood than in single-stranded DNA. Presently, adducts in white blood cells can be detected after exposure of human blood to sulfur mustard concentrations $2 μM (van der Schans et al. 1994). Another mustard gas metabolite, N7-(2-hydroxyethylthioethyl)-guanine, has been detected in the urine of guinea pigs exposed to mustard gas (Fidder et al. 1996a).

Van der Schans et al. (1994) synthesized N7-HETE-GMP for use as a hapten to generate monoclonal antibodies against the major adduct, N7-[2-[(hydroxyethyl)thio]ethyl]guanine (N7-HETE-Gua), formed after alkylation of DNA with mustard gas. Six stable clones producing antibody specific for mustard gas adducts were isolated from immunized mice and characterized by ELISA. These antibodies have potential in the development of a single-cell assay with immunofluorescence microscopy to quantify adduct formation in skin exposed to mustard gas.

To enable detection of low-level exposure to mustard gas, mustard gas adducts with proteins have also been explored. Mustard gas alkylates hemoglobin (Black et al. 1997a, 1997b; Fidder et al. 1996a; Noort et al. 1996, 1997) and albumin (Noort et al. 1999). In hemoglobin, the N-terminal valine, on both the α and β chains, and histidine residues were identified as key sites of interaction (Black et al. 1997a, 1997b; Noort et al. 1997). A cysteine residue of albumin was identified as a site of mustard gas alkylation (Noort et al. 1999). A procedure employing gas chromatography-mass spectrometry with modified Edman degradation has been developed for the determination of the adduct of mustard gas with the N-terminal valine residue of hemoglobin (Fidder et al. 1996a). A mass spectrometric analysis of the adduct of mustard gas with the cysteine residue of albumin, S-[2-[(hydroxyethyl)thio]ethyl]Cys-Pro-Phe, provided a detection limit for mustard gas an order of magnitude lower than the modified Edman assay for hemoglobin (Noort et al. 1999). The drawback for albumin adduct detection is the faster elimination rate. The half-life of albumin is 20–25 days versus the 120 day life span of hemoglobin. Both protein adducts have been detected in the blood of two victims of the Iran-Iraq War using the respective assay (Benschop et al. 1997; Noort et al. 1999).

3.8.2 Biomarkers Used to Characterize Effects Caused by Mustard Gas

An antibody that binds mustard gas has been developed as a tool for research and forensic detection (Lieske et al. 1992). The antibody was assessed by testing the cross-reactivity of rabbit anti-mustard gas antiserum with mustard gas and related compounds. The antiserum was inhibited to a similar degree by mustard gas, chloroethyl ethyl sulfide (CEES), and chloroethyl methyl sulfide (CEMS). Single arm nitrogen mustard produced 60% less inhibition. Thiodiglycol, the principal hydrolysis product of mustard gas, does not react with the antibody.

Myeloperoxidase (MPO) activity was measured to characterize the dose- and time-dependence of polymorphonuclear leukocyte (PMN) infiltration during the development of mustard gas lesions on the

skin of euthymic hairless guinea pigs (HGP) (Bongiovanni et al. 1993). PMNs, as assessed by MPO levels, peaked at 9 hours postexposure, irrespective of mustard gas vapor dose. At 9 hours, a maximum 20-fold increase in PMNs was measured relative to control sites. At 24 hours postexposure, MPO levels dropped to twice control levels. Because a 9-hour postexposure period coincides with epidermal detachment characterized by electron microscopy, these results suggest that PMNs participate in the HGP cutaneous inflammatory response to mustard gas dermal exposure and that MPO may be a useful biological marker for evaluating putative antivesicants.

Electrophoretic protein separation of soluble homogenate fraction of full thickness skin samples from weanling pigs exposed one time dermally to mustard gas, revealed an acute increase in an approximately 160 kDa protein band, consistent with an increase in haptoglobin (Blank et al. 1996). The amount of 160 kDa protein was related to the extent of vascular damage and may be an indicator of the severity of tissue damage. Elevated serum haptoglobin levels have been reported in rabbits exposed to hemi-mustard, but it is unknown whether any nonmustard chemicals induce a similar increase.

Pu et al. (1995) have shown that interleukin 1α (IL-1α) can be used to quantify the cytotoxicity resulting from mustard gas-induced damage to cellular DNA. A high correlation was observed between the dose-responsive increase in the amount of IL-1α in cultured human epidermal keratinocytes (HEK) after exposure to mustard gas, measured using a monoclonal antibody to human IL-1α, and the fraction of viable cells, assessed by trypan blue exclusion. The mustard gas dose-responsive increase in DNA crosslinking immediately after exposure, measured by ethidium bromide intercalation, correlated with the increase in cellular IL-1α 72 hours after exposure and was, therefore, predictive of delayed cytotoxicity in the exposed culture.

Inhalation exposure of rats to mustard gas by assay of lung lavage fluid revealed a time-dependent increase in proteolytic activity (Cowan et al. 1997). Lindsay et al. (1996) have shown that the serine protease inhibitor mafenide HCL and cysteine protease inhibitor E64 prevent the dermal-epidermal separation in human skin explants after mustard gas exposure. Compounds that reduce mustard gas-induced proteolytic activity have potential in elucidating the mechanisms of mustard gas toxicity.

3.9 INTERACTIONS WITH OTHER CHEMICALS

No data were located on the interactions of mustard gas with other toxicants likely to be found at hazardous waste sites.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to mustard gas than will most persons exposed to the same level of mustard gas in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of mustard gas, or compromised function of organs affected by mustard gas. Populations who are at greater risk due to their unusually high exposure to mustard gas are discussed in Section 6.7, Populations With Potentially High Exposures.

Humans show varying degrees of sensitivity to mustard gas (Renshaw 1946; Sulzberger et al. 1947). For dermal contact, fair-skinned people are more sensitive than dark-skinned people. These reports also indicate that individuals with previous exposure are more sensitive to the dermal effects of mustard gas. It is possible that individuals with respiratory problems (asthma, emphysema, etc.) might be more sensitive to the effects of mustard gas and might suffer acceleration of their disease following exposure. Since mustard gas has been associated with lung cancer, people who smoke may be at greater risk.

Children are more susceptible to the effects of mustard gas than adults. The time of onset of mustard gas manifestations in children is shorter and the severity of the lesions is higher than in adults, possibly due to more delicate skin and epithelial tissues (Momeni and Aminjavaheri 1994). Cough and vomiting were the first symptoms in children, but not in adults. Children had higher occurrences of ocular, respiratory, and gastrointestinal effects than adults. Genital manifestations were less frequent in children and teenagers than adults, whereas other skin lesions had no apparent age-relation.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to mustard gas. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to mustard gas. When

specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to mustard gas:

Augerson WS, Sivak A, Marley WS. 1986. Chemical casualty treatment protocol development - treatment approaches. Vol II-IV. Cambridge, MA: Arthur D. Little, Inc.

Marrs TC, Maynard RL, Sidell FR. 1996. Chemical warfare agents. John Wiley & Sons, New York.

OPCW. 2001. Organization for the prohibition of chemical weapons, decontamination of chemical warfare agents. Http://www.opcw.nl/chemhaz/decon.htm. March 13, 2001.

SBCCOM. 2001. Material safety data sheet, mustard gas. Aberdeen Proving Ground, MD: U.S. Army Soldier and Biological Chemical Command. Http://in1.apgea.army.mil/RDA/msds/hd.htm. March 13, 2001.

U.S. Army. 1995. Treatment of chemical agent casualties and conventional military chemical injuries. Washington, DC: U.S. Department of the Army, FM 8-285. Http://www.adtdl.army.mil/cgi-bin/atdl.dll/query/info/FM+8-285. March 22, 2001.

Willems JL. 1989. Clinical management of mustard gas casualties. Annales Medicinae Militaris Belgicae, 1989, Vol 3 supp. Heymans Institute of Pharmacology, University of Ghent Medical School and Royal School of the Medical Services, Leopoldskazerne, B-900 Ghent, Belgium.

3.11.1 Reducing Peak Absorption Following Exposure

Decontamination procedures should be initiated immediately after exposure. The eyes should be washed immediately with water for at least 15 minutes, even if no symptoms are present, since it is known that ocular and dermal symptoms are delayed (Dreisbach and Robertson 1987; Goldfrank et al. 1990; Solberg et al. 1997). Of the many fluids studied for eye irrigation, none has proved more effective than tap water (Solberg et al. 1997). Contaminated clothing should be removed and the skin should be decontaminated. Decontamination should include the groin, axillae, and perineal areas. Rapid removal from skin is critical, as mustard gas penetrates skin within minutes of exposure. Topical decontamination with hypochlorite solutions was examined in euthymic hairless guinea pigs (Gold et al. 1994) and rabbits (Hobson et al. 1993). No significant wound differences were found between water only and various concentrations of hypochlorite and high concentrations were irritating to the skin; however, decontamination with a 0.5% solution is standard in many military medical systems. It has been suggested that removal of mustard gas with water alone is contra-indicated as mustard gas spreads over more skin surface and increases the area of blistering (Kumar et al. 1991). Absorbent powders such as calcium chloride may be sprinkled onto the exposed skin, allowed to absorb the mustard gas, and then

washed off with water (Solberg et al. 1997). In a study in which Fuller's earth (FE), N,N'-dichloro-bis (2,4,6-trichlorophenyl) urea (CC-2), and their various combinations (w/w ratios) were evaluated for their decontamination efficacy against mustard gas when applied on mouse skin, maximum protection was obtained with FE and CC-2 in a combination of 80:20 (w/w) (Kumar et al. 1991). The currently fielded ARMY skin decontamination kit (SDK) is the M291 SDK, which contains XE-555 resin (Amberguard 555) powder as the chief component (SBCCOM 2001).

3.11.2 Reducing Body Burden

Currently, there is no proven therapy against mustard gas. Mortality can be reduced by administration of electrolyte solutions by mouth, subcutaneously, or intraperitoneally commencing early and continuing throughout the intoxication period (Cullumbine 1947). Electrolyte replacement is needed due to losses from skin locally, and in the intestine and via saliva, vomitus, and diarrheaic stools. In mice a single dose of saline or glucose-saline (5 mg glucose/kg) offered protection to mice after topical mustard gas exposure; survival was 83% with saline treatment compared to 33% without treatment (Sugendran et al. 1994). In severely injured victims, systemic analgesics should be started after examination. Patients whose ocular injuries are limited to the conjunctiva require no additional treatment subsequent to irrigation. Corneal lesions may be detected by staining with fluorescein and examination with blue light. Treatment for injury to the cornea should include daily irrigation, mydriatics to ease the eye pain produced by spasm of the ciliary muscle and to prevent posterior iridolenticular adhesions, antibiotic drops to prevent secondary bacterial infections, local medications to control intraocular pressure, and systemic analgesics (Solberg et al. 1997). In cases of ocular injury, local anaesthetic drops should be avoided other than for ophthalmologic examination, as they are toxic to both healthy and damaged corneas. Although recommended, the use of sterile petroleum jelly to prevent the lid margins from sticking together should be delayed until after sufficient irrigation, since mustard gas will dissolve and concentrate in the jelly (Solberg et al. 1997). Ocular bandages should not be applied as they might raise the corneal temperature and increase the toxic effects (Solberg et al. 1997).

Patient care should include supportive treatment protocols for skin injury, respiratory distress, and cardiac dysrhythmias (Dreisbach and Robertson 1987; Haddad and Winchester 1990). There may be a delay of onset of toxicity in exposed individuals. Severe respiratory distress may be delayed for up to 72 hours depending on the concentration and duration of exposure (Ellenhorn and Barceloux 1988). In

cases of damage to the upper respiratory tract, antibiotic cover is recommended to prevent infection (Murray and Volans 1991).

Faster healing and less scarring have been reported when blisters were drained. While aseptic procedures are prudent for handling all bodily fluids, there are conflicting reports as to the danger of the blister fluid itself. There are no reports of mustard gas detected in blister fluid (Jakubowski et al. 2000); however, secondary blistering running proximal to an original blister, thought to be due to leaking fluid, was reported in a case of accidental exposure during destruction of mustard gas stockpiles (Bide et al. 1993).

Delayed keratitis should be treated with ocular lubricants, therapeutic lenses, and in severe cases, tarsorrhaphy (Solberg et al. 1997). Keratoplasty should be considered if there is significant opacification of the cornea accompanied by deposition of crystals and cholesterol.

In a study of the mustard gas vesication following pretreatment with topically applied agents, the most promising composition was comprised of petrolatum, sorbitan stearate, and water with either of the N-halo oxidants 1,3,4,6-tetrachloro-7,8-diphenyl-2,5-diiminoglycoluril (S-330) or 1,3-dichloro-5-5-dimethylhydantoin, and optionally, with a barrier-providing polymer such as perfluoroalkylpolyether (FOMBLIN HC/04, HC/25, or HC/R) or a polysiloxane (Kwong 1996). A topical skin protectant cream containing perfluoroalkylpolyether and polytetrafluoroethylene, ICD 2289, being developed to protect service members from exposure to chemical warfare agents, was shown to reduce the mustard gas-induced lesion area to 18% of untreated lesion area when applied as a pretreatment in rabbits (Liu et al. 1999). Canadian Reactive Skin Decontamination Lotion (RSDL), which is a 1.25 molal solution of potassium 2,3-butanedionemonoximate (KBDO) in 9:1 polyethyleneglycol monoethylether (500 nominal weight):H_2O, was shown to reduce the severity and scarring of mustard gas-induced lesions on the shaved back of guinea pigs (Bide et al. 1993). Also reported was the case of an employee who suffered minor mustard gas burns to the wrist and forearm during destruction of mustard gas stockpiles at the Canadian Defense Research Establishment Suffield (DRES). Treatment was carried out partly at DRES and partly at a local hospital. One set of burns received treatment with RSDL at DRES where it was available and another set did not as RSDL was not available at the local hospital. The blister initially without RSDL treatment burst and a series of secondary burns running proximal to the original blister formed. The RSDL treated burn was much less severe and no secondary burns formed.

An exciting new destructive absorption technology (DAT) employs highly reactive nanoparticles (RNP) to neutralize toxic substances including mustard gas. Preliminary studies indicate that RNP remain active against chemical agents when incorporated into a base cream and are compatible with skin contact (Koper et al. 1999).

Pulsed carbon dioxide (CO_2) laser debridement has been shown to be effective in clearing the epidermis of mustard gas damaged cells (Smith et al. 1997b). In weanling pigs, whose skin was exposed to mustard gas, CO_2 laser debridement of the exposed skin resulted in clearing of the cytologic atypia, reduced inflammatory infiltrate, and increased numbers of stromal cells within the papillary dermis. At 14 days postexposure, there was no significant difference between skin laser-debrided at 6, 24, or 48 hours after exposure.

Animal experiments have shown that sodium thiosulfate, N-acetyl-L-cysteine, nicotinamide, nicotinic acid, promethazine, dexamethasone, prednisone and vitamin E have decreased tissue damage, but their efficacy in humans is not known (Dabney 1991; Papirmeister et al. 1991; Vojvodic et al. 1985). Thiosulfate likely acts as a mustard scavenger, vitamin E as an antioxidant, and the corticosteroids by inhibiting lipooxygenase activity leading to synthesis of prostaglandins and leukotrienes (Borak and Sidell 1992). Application of provodine iodine (PI) ointment to the shaved back of guinea pigs, up to 10 minutes following mustard gas exposure has been shown to provide significant protection from ulceration (Wormser et al. 1997). Histopatholical evaluation of PI-treated skin showed only moderate thickening of the epidermis with slight hyperkeratosis, whereas deep epidermal ulceration involving the superficial dermis was evident without PI treatment. In a comparative study of chemical burn therapies in guinea pigs, debridement with trypsin-linked gauze (Debridase) was more effective in reducing the lesion area than surgical excision or laser ablation (Eldad et al. 1998b).

In guinea pigs injected intratracheally with mustard gas, subsequent treatment with betamethasone, a glucocorticoid, significantly increased tracheal epithelium height by about 20% and cell density compared to untreated animals (Calvert et al. 1996). Superoxide dismutase was effective in reducing the lesion area when administered before, but not after topical application of mustard gas in guinea pigs (Eldad et al. 1998a).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Mustard gas is thought to induce structural changes in cellular DNA, as indicated by altered dye response in flow cytometric studies (Smith et al. 1993). Reducing or preventing the ability of mustard gas to alkylate DNA and critical target molecules will reduce toxicity. Reduction of target structural changes may by possible by the use of compounds that react with or scavenge mustard gas and lower target alkylation levels. The speed at which mustard gas reacts presents a difficulty to this strategy of treatment.

Cell cycle kinetics are involved in the cytotoxic processes following mustard gas exposure. Mustard gas-induced damage at subvesicating concentrations (<50 μM) to genomic DNA in cultured HEK resulted in a dose-related reversible block at the G_2/M phase of the cell cycle (Smith et al. 1993). Okadaic acid and calyculin A, inhibitors of protein phosphatase 2A (PP2A) completely reversed the mustard gas-induced G_2/M block (Hart and Schlager 1997). Exposure of human peripheral blood lymphocytes (PBL) to vesicating equivalent concentrations of mustard gas ($50 μM) resulted in irreversible blockage at the G1/S interface (Smith et al. 1998). DNA became terminally fragmented. Compounds might be used to hold cells in a selected phase in order to permit DNA repair processes to correct the damaged DNA before normal proliferative events are allowed to proceed. Mimosine, one such inhibitor, was shown to provide limited protection against cytotoxicity of vesicating equivalenet concentrations of mustard gas in HEK and HeLa cells (Smith et al. 1998).

Reversal of secondary consequences of alkylation requires a better understanding of the biochemical pathways of toxicity and may require interventions for more than one mechanism of action. As pointed out by Papirmeister et al. (1991), this strategy would provide temporary measures, slowing down the injury process and buying time for intracellular repair processes, thereby avoiding the simultaneous necrosis of massive numbers of cells as occurs in mustard gas-induced epithelial lesions. Tissue function may remain close to normal if cell death can be spread out over a sufficiently long period of time, and dead cells are replaced through endogenous tissue repair and regeneration mechanisms.

Sawyer et al. (1996) examined the possibility that the toxicity of mustard gas is due to the induction or activation of nitric oxide synthase (NOS). L-nitroarginine methyl ester (L-NAME), an inhibitor of NOS, was found to confer protection to primary cultures of chick embryo forebrain neurons against the toxicity of mustard gas when administered as a pretreatment or up to 3 hours postexposure. While NOS requires

L-arginine as a substrate, mustard gas toxicity and L-NAME protection were independent of L-arginine concentration. Thus, the role of NOS in mustard gas toxicity remains unclear but L-NAME appears to have potential as a therapeutic drug.

Niacinamide (750 mg/kg, intraperitoneal), while not effective as a postexposure treatment, did inhibit microvesicle formation by 50% after cutaneous exposure to mustard gas in hairless guinea pigs when given as a 30-minute pretreatment (Yourick et al. 1991). In this study, when niacinamide was administered as 30-minute pretreatment, NAD^+ content in mustard gas treated skin biopsies decreased to about 40% of control levels. When niacinamide was administered at two times, both as a 30-minute pretreatment and as a 2-hour treatment, NAD^+ was maintained at control levels, but microvesicle formation was about the same as in the pretreatment only case, indicating that maintaining skin NAD^+ content did not absolutely confer protection from microvesication, nor was it a necessary factor for preventing microvesication.

Fpg protein is thought to protect cells from toxicity by removing ring-opened N-7 guanine adducts from DNA. Li et al. (1997) investigated the action of Fpg protein on the ring-opened form of the mustard gas adduct 7-hydroxyethyl-thioethylguanine (ro-HETEG). Fpg protein released ro-HETEG from DNA modified by [14C]sulfur mustard in an enzyme- and time-dependent manner, and may offer some protection against the toxic action of mustard gas.

The toxic effects of sulfur mustard have been attributed to DNA modification with the formation of 7-hydroxyethylthioethyl guanine, 3-hydroxyethylthioethyl adenine and the cross-link, di-(2-guanin-7-yl-ethyl)sulfide. Bacterial 3-methyladenine DNA glycosylase II (Gly II) was found to releases both 3-hydroxyethylthioethyl adenine and 7-hydroxyethylthioethyl guanine from calf thymus DNA was modified with [14C]sulfur mustard, suggesting that glycosylase action may play a role in protecting cells from the toxic effects of mustard gas (Matijasevic et al. 1996).

3.12 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of mustard gas is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to

assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of mustard gas.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

Acute-duration inhalation and acute- and intermediate-duration oral MRLs are derived from animal data. There is a greater need for additional chronic inhalation and dermal data over oral data as mustard gas hydrolyzes in water, and oral exposure is the least likely of the three routes. Laboratory animals with fur do not provide optimal models for dermal exposure as they do not have sweat glands on most of their body. Further exploration of relevant models including human skin grafts, porcine skin flaps in explant culture, and hairless guinea pigs is prudent to study the biochemical events in mustard gas toxicity and identify effective therapies.

Questions still remain regarding the mechanisms of toxicity of mustard gas. The database would benefit from research leading to greater understanding of the following (Papirmeister et al. 1993):

- The involvement of apoptotic and necrotic cell death processes to the cytotoxic and acute skin injurant actions of mustard gas.
- The importance of DNA repair and cell cycle traverse in skin cells that undergo apoptosis leading to lesion formation.
- The reason PADPRP inhibitors prevent losses of NAD^+, ATP, and viability in mustard gas-treated human peripheral blood lymphocytes (PBL), but fail to prevent mustard gas-induced cytotoxicity in HEK or mustard gas-induced acute skin injury.
- Any pathways, other than the PADPRP-mediated NAD^+ loss, by which mustard gas-induces inhibition of glycolysis and energy depletion in HEK.
- The mechanism(s) responsible for increasing and maintaining high levels of intracellular calcium in mustard gas exposed cells.
- Relationships between mustard gas and protein regulation in connection with vesication.
- The contribution of reactive oxygen species to mustard gas cytotoxicity.

- The role of inflammation in the development of the acute cutaneous mustard gas injury.

As our understanding of these mechanisms increases, more research is needed to identify therapeutic countermeasures.

3.12.1 Existing Information on Health Effects of Mustard Gas

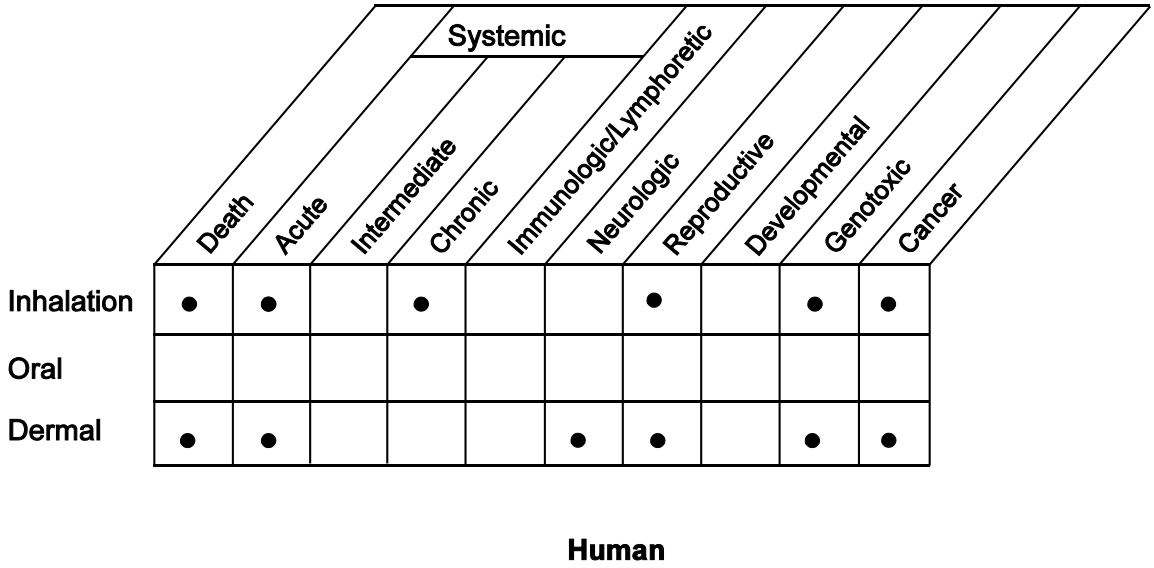
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to mustard gas are summarized in Figure 3-4. The purpose of this figure is to illustrate the existing information concerning the health effects of mustard gas. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Data are available for humans regarding respiratory disease and cancer, and the deaths caused by these diseases following acute and chronic inhalation exposure. Very limited animal data are available regarding death, developmental and reproductive effects, and cancer following inhalation exposure. There are no data available on the toxicity of mustard gas from oral exposure in humans. Data are available for animals regarding acute and subchronic toxic effects following oral exposure. Limited data are available in humans and animals regarding skin effects from dermal exposure, and cancer in humans from dermal exposure.

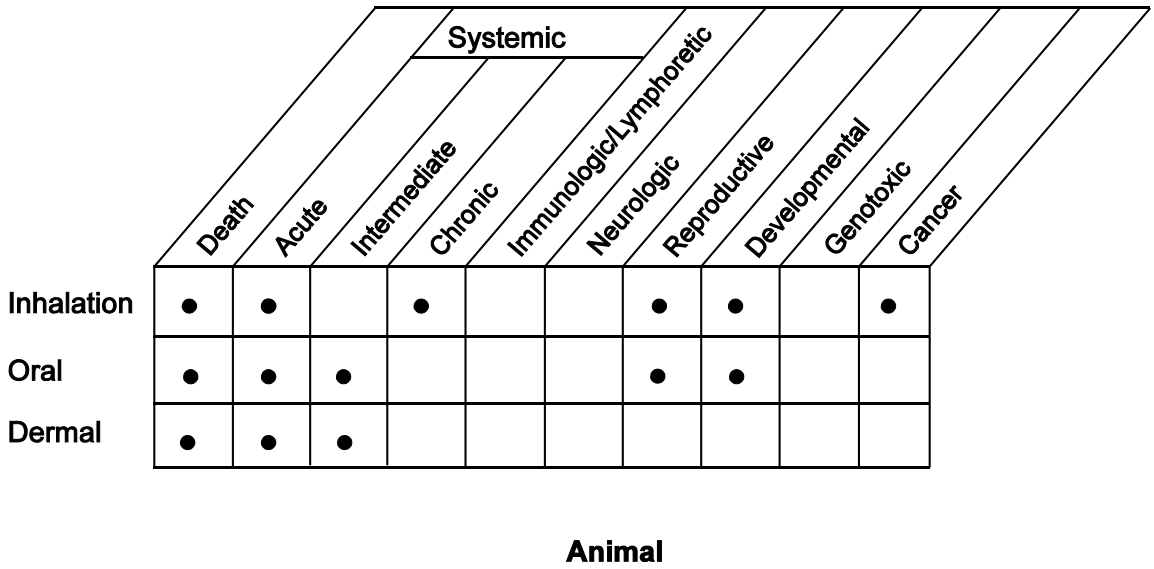
3.12.2 Identification of Data Needs

Acute-Duration Exposure. Sufficient information is available from human exposure data to identify the skin (Frank 1967; Jakubowski et al. 2000; Momeni and Aminjavaheri 1994; NRC 1985; Renshaw 1946; Sinclair 1948, 1950; Smith et al. 1919; Sulzberger et al. 1947; Wulf et al. 1985) and respiratory passages (Beebe 1960; Case and Lea 1955; Momeni and Aminjavaheri 1994; Momeni et al. 1992; Norman 1975) as target organs from acute exposure to this airborne chemical warfare agent. Data from animal studies also suggest that acute exposure to mustard gas is harmful to the gastric mucosa (DOA 1987b), skin (Chauhan et al. 1993a, 1993b, 1995; McAdams 1956; Venkateswaran et al. 1994;

Figure 3-4. Existing Information on Health Effects of Mustard Gas



Human



Animal

● Existing Studies

Vogt et al. 1984; Young 1947) and respiratory passages (Allon et al. 1993; Heston 1953b; Vijayaraghavan 1997; Winternitz and Finney 1920). Since mustard gas has been used in combat, it is known to be lethal from primary or secondary effects (Case and Lea 1955; Sinclair 1948, 1950; Somani and Babu 1989). While no human oral data are available, effects to the gastric mucosa would be expected as mustard gas is a vesicant and direct alkylating agent. Acute inhalation and oral MRLs have been derived.

Intermediate-Duration Exposure. Intermediate-duration exposure during combat has shown that mustard gas can be lethal. Wartime and occupational studies in humans have identified the skin (Bullman and Kang 2000; NRC 1985; Sinclair 1948, 1950; Wulf et al. 1985) and respiratory passages (Bullman and Kang 2000; Case and Lea 1955; Easton et al. 1988; Nishimoto et al. 1970; Somani and Babu 1989) as the target organs for mustard gas for intermediate-duration exposure. Data from animal studies also suggest that subchronic exposure to mustard gas is harmful to the gastric mucosa (Sasser et al. 1996a, 1996b). While no human oral data are available, effects to the gastric mucosa would be expected as mustard gas is a vesicant and direct alkylating agent. An intermediate-duration oral MRL has been derived. Additional studies are required in order to derive an intermediate-duration inhalation MRL. Male dominant lethal studies in animals with exposure by the inhalation and dermal routes including site of application histological examinations would provide valuable data. It seems likely that as with the oral route, the application site would be more sensitive to the effects of mustard gas than the male reproductive system; however, when considering combat exposure, the perineal area was frequently a site of application.

Chronic-Duration Exposure and Cancer. Epidemiological studies of mustard gas workers have identified the skin (Inada et al. 1978; Klehr 1984; NRC 1985) and respiratory system (Easton et al. 1988; Manning et al. 1981; Morgenstern et al. 1947; Nishimoto et al. 1970; Somani and Babu 1989; Tokuoka et al. 1986; Wada et al. 1968; Weiss and Weiss 1975; Yamada 1963; Yamakido et al. 1996) as the target organs. Chronic inhalation and oral MRLs were not derived due to the lack of quantifiable exposure data. In order to derive an MRL, additional studies with quantified exposure amounts and durations would be needed for both routes.

Factory workers who have been exposed to undetermined levels of mustard gas for a number of years have been shown to develop respiratory cancer (Easton et al. 1988; Manning et al. 1981; Morgenstern et al. 1947; Nishimoto et al. 1970; Tokuoka et al. 1986; Wada et al. 1968; Weiss and Weiss 1975; Yamada

1963; Yamakido et al. 1996). In order to develop cancer effect levels, appropriate animal studies would be necessary since there are no adequate studies currently available.

Genotoxicity. Mustard gas is known to be highly genotoxic *in vitro*, and further studies would likely not alter this conclusion (Ashby et al. 1991; Auerbach 1946; Ball and Roberts 1971/72; Capizzi et al. 1974; Fahmy and Fahmy 1971, 1972; Fan and Bernstein 1991; Ichinotsubo et al. 1977; Kircher and Brendel 1983; Lin et al. 1996a, 1996b; Ludlum et al. 1994; Ribeiro et al. 1991; Scott et al. 1974; Venitt 1968; Venkateswaran et al. 1994; Walker and Thatcher 1968).

Reproductive Toxicity. Several human and animal studies suggest that mustard gas affects the male reproductive function (Azizi et al. 1995; Graef et al. 1948; McNamara et al. 1975; Pour-Jafari and Moushtagi 1992; Rozmiarek et al. 1973; Sasser et al. 1993). Data from animal studies regarding oral exposure to mustard gas indicate that the acute-duration oral MRL derived within this profile would be protective of this system. Additional acute and chronic inhalation and oral studies are required to determine exposure levels for these routes and durations that would limit reproductive toxicity.

Developmental Toxicity. In animal studies, fetal toxicity was evidenced by reduced body weight and ossification. Data from animal studies regarding oral exposure to mustard gas indicate that the acute-duration oral MRL derived within this profile would be protective of fetal development. Additional chronic oral and acute and chronic inhalation studies are required to determine exposure levels for these routes and durations that would limit fetal toxicity.

Immunological and Lymphoreticular Toxicity. Mustard gas-induced damage to the lymph system was found in war casualties and in animals studies following inhalational, oral, or dermal exposure. Mustard gas-induced lymphoreticular toxicity does not appear to be route- or species-specific. Data from animal studies regarding inhalation and oral exposure to mustard gas indicate that the acute-duration inhalation and oral MRLs derived within this profile would be protective of the lymph system. Additional chronic inhalation and oral studies are required to determine exposure levels for these routes that would limit lymphoreticular toxicity.

Neurotoxicity. Only minimal animal data are available regarding the neurotoxicity of mustard gas (Alexander 1947; Sasser et al. 1993; Winternitz and Finney 1920). Chronic or latent pain in the exposed skin area experienced by victims of mustard gas attacks suggests that mustard gas may cause persistent damage to the afferent nerve system (Thomsen et al. 1998). This effect appears specifically related to dermal exposure and additional studies are required to determine protective exposure limits. Pharmacokinetic data are insufficient to judge the potential for mustard gas to affect this system.

Epidemiological and Human Dosimetry Studies. Two types of human epidemiology studies are available: those using men who were exposed briefly during combat in World War I (Beebe 1960; Case and Lea 1955; Norman 1975; Sinclair 1948, 1950), and those exposed for a longer period when producing mustard gas in Japanese (Nishimoto et al. 1970, 1983; Tokuoka et al. 1986; Wada et al. 1968; Inada et al. 1978; Yamada 1963; Yamakido et al. 1996), German (Weiss and Weiss 1975), British (Easton et al. 1988; Manning et al. 1981), or American factories (Bullman and Kang 2000). In these studies, exposure duration and levels were not quantified, although a relation to dose is suggested since deaths due to lung cancer increased with greater likelihood of exposure or service years in factories. Continued monitoring of mustard gas victims of the Iran-Iraq War would provide valuable toxicity information.

Biomarkers of Exposure and Effect.

Exposure. Two closely related metabolites of mustard gas not detected in normal urine, 1,1'-sulphonylbis[2-(methylsulphinyl)ethane] and 1-methylsulphinyl-2-[2-methylthio)ethyl-sulphonyl]ethane, have been detected in urine collected from Iran-Iraq War casualties of mustard gas poisoning (Black and Read 1995b; Black et al. 1991). Mustard gas has also been shown to alkylate hemoglobin (Black et al. 1997a, 1997b; Fidder et al. 1996a; Noort et al. 1996, 1997) and albumin (Noort et al. 1999). Both protein adducts have been detected in the blood of Iran-Iraq War victims (Benschop et al. 1997; Noort et al. 1999). Development and validation of standard assays for these urine metabolites and blood protein adducts would be valuable tools for retrospective detection of exposure.

Effect. Various local enzymatic activity and protein alterations have been reported in connection with mustard gas exposure, thus providing potential as biomarkers of effect. Additional research providing a further understanding of the mechanisms of mustard gas toxicity is required before assay validation.

Absorption, Distribution, Metabolism, and Excretion. There is a substantial toxicokinetic database for intravenous and intraperitoneal routes of mustard gas exposure in animals. These data indicate that it can be absorbed (Cameron et al. 1946; Cullumbine 1946, 1947; Drasch et al. 1987; Hambrook et al. 1993; Klain et al. 1991; Langenberg et al. 1998; Nagy et al. 1946; Papirmeister et al. 1984a, 1984b; Renshaw 1946; Smith et al. 1919) and is excreted in the urine (Black et al. 1992a, 1992b; Davison et al. 1961; Hambrook et al. 1992; Jakubowski et al. 2000; Maisonneuve et al. 1993; Roberts and Warwick 1963; Sandelowsky et al. 1992; Smith et al. 1958; Wils et al. 1985, 1988). Langenberg et al. (1998) detected mustard gas DNA adducts in tissues following inhalation exposure in guinea pigs. Metabolic pathways are presumed based on these data. As the route of exposure appears to be an important toxicokinetic factor, more studies would be helpful to adequately characterize the rate and extent of mustard gas absorption, distribution, and excretion via the dermal and inhalation routes.

Comparative Toxicokinetics. Data are available to indicate that the skin, respiratory tract, male reproductive system, and lymph nodes are targets in both humans and animals. Since humans do not have the fur that most laboratory animals do, and since humans have sweat glands over most of their body whereas animals do not, human responses to skin irritants such as mustard gas are different from those of animals. The hairless guinea pig model has been used to study the biochemical events in sulfur mustard toxicity. Toxicokinetic studies in animals (rats, mice, and pigs) (Black et al. 1992a, 1992b; Davison et al. 1961; Fidder et al. 1996a; Hambrook et al. 1992; Roberts and Warwick 1963; Sandelowsky et al. 1992; Smith et al. 1958), and humans (Benschop et al. 1997; Black and Read 1995b; Black et al. 1991; Jakubowski et al. 2000; Noort et al. 1999; Wils et al. 1985) indicate that the metabolites are similar across species.

Methods for Reducing Toxic Effects. Based on current concepts regarding the mechanisms of toxicity of mustard gas, compounds with known biochemical or cellular actions can be identified that may interfere with some or all of pathways of toxicity. Additional studies providing a more thorough mechanistic understanding, identification of additional pathway affectors, and validation of the efficacy of existing compounds would be valuable.

Children's Susceptibility. Besides two reports of accidental deaths of children exposed to mustard gas, clinical reports of children exposed during the Iran-Iraq War provide the only nonlethal effects data in children. The main exposure pathways for children are the same as for adults. The time of onset of mustard gas manifestations is shorter and the lesion severity greater in children than in adults, possibly

due to more delicate skin and epithelial tissues. Children's susceptibility to the effects of mustard gas is likely correlated to their understanding of the need for precautionary measures, ability to recognize exposure, and initiate decontamination.

3.12.3 Ongoing Studies

One of the major goals of future medical chemical defense research on vesicants is the search for effective prophylactic and therapeutic countermeasures. Screening programs exist for candidate antidotes.

Ongoing studies pertaining to mustard gas have been identified and are shown in Table 3-4.

Table 3-4. Ongoing Studies on Health Effects of Mustard Gas

Investigator	Affiliation	Research description	Study sponsor
Back, DD	Mainstream Engineering Corporation Rockledge, Florida	Highly destructive polymer-contained neutralizing skin protectants: Feasibility of coated topical skin protectant additives using a new class of reactive metal alloys	Army
Hendler, FJ MD, PhD	Department of Veterans Affairs Louisville, Kentucky	Effect of hazardous substances on reproductive capacity and developmental abnormalities	Department of Veterans Affairs Washington, DC
Hinshaw, DB MD	Department of Veterans Affairs Ann Arbor, Michigan	The cytoskeleton and ATP in sulfur mustard-mediated injury to endothelial cells and keratinocytes	Department of Veterans Affairs Washington, DC
Kang, HK DPH	Department of Veterans Affairs Washington, DC	Mortality follow-up of veterans exposed to mustard gas in World War II	Department of Veterans Affairs Washington, DC
Klabunde, KJ	Nantek, Inc. Manhattan, Kansas	Development of reactive topical skin protectants against sulfur mustard and nerve agents	Army
Myer, SB	Tienzyme, Inc. State College, Pennsylvania	Use of fungal peroxidases for neutralization of mustard gas	Army
Richmond, A PhD	Department of Veterans Affairs Nashville, Tennessee	The role of chemokines in wound healing and sepsis:chemical burn (sulfur mustard) model of injury	Department of Veterans Affairs Washington, DC
Sweeney, JF MD	Department of Veterans Affairs Ann Arbor, Michigan	Regulation of polymorphonuclear-leukocyte (PMN) survival and function by proinflammatory agents that are released as a consequence of sulfur mustard mediated injury	Department of Veterans Affairs Washington, DC

Source: FEDRIP 2001

4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

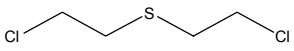
Information regarding the chemical identity of mustard gas is located in Table 4-1. Mustard gas has several synonyms; the most common are sulfur mustard, “H”, and “HD”. “H” refers to undistilled or raw mustard gas, which contains a large fraction of impurities (see Table 4-2). “HD” refers to a distilled or purified form of mustard gas (see Table 4-3). “HT” is often called mustard gas even though it is a mixture of 60% “HD”, <40% Agent T (bis[2-(2-chloroethylthio)ethyl]ether, CAS# 63918-89-8), and a variety of sulfur contaminants and impurities. Most studies on mustard gas are based on its distilled or purified form, “HD” (Munro et al. 1999).

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of mustard gas (HD) is located in Table 4-4.

Weapons-grade mustard gas can contain stabilizers, starting materials, or by-products formed during manufacturing, and products formed from slow reactions during storage (Munro et al. 1999). The typical compositions of HD and H are illustrated in Tables 4-3 and 4-4, respectively (NRC 1999; Rosenblatt et al. 1996). In general, a residual “heel” (i.e., a gel that with not flow) forms with the ageing of mustard gas. The heel can amount to more than 10% of the agent and usually contains 14–53% mustard gas, 42–86% cyclic sulfonium ions, and also metals, such as iron sulfide (NRC 1999).

Table 4-1. Chemical Identity of Mustard Gas

Characteristic	Information	Reference
Chemical name	Bis(2-chloroethyl) sulfide	HSDB 2001
Synonym(s)	β,β'-Dichloroethyl sulfide; β,β'-Dichloroethyl sulphide; 1-Chloro-2-(β-chloroethylthio)ethane; 1,1'-Thiobis(2-chloroethane); 2,2'-Dichloro-diethyl sulfide; 2,2'-Dichlorodiethyl sulphide; 2,2'-Dichloroethyl sulphide; 2,2'-Dichloroethyl sulfide; Bis(β-chloroethyl)sulfide; Bis(β-chloro-ethyl)sulphide; Bis(2-chloroethyl)sulphide; Di-2-chloroethyl sulfide; Di-2-chloroethyl sulphide; Dichloro-diethyl-sulphide; Dichloro-diethyl sulfide; Dichloroethyl sulfide; Diethyl sulfide, 2,2'-dichloro; Distilled mustard; Ethane, 1,1'-thiobis(2-chloro-; Gelbkreuz; H; HD; Kampstoff "Lost"; Lost; Mustard, sulfur; Mustard vapor; Mustard gas; Mustard HD; S mustard; S-lost; S-Lost; S-yperite; Schwefel-Lost; Senfgas; Sulfide, bis(2-chloroethyl); Sulfur mustard gas; Sulfur mustard; Yellow Cross Liquid; Yellow Cross Gas	HSDB 2001
Registered trade name(s)	No data	
Chemical formula	$C_4H_8Cl_2S$	Budavari et al. 1996
Chemical structure		IARC 1975; Budavari et al. 1996
Identification numbers:		
CAS registry	505-60-2	HSDB 2001
NIOSH RTECS	WQ0900000	HSDB 2001
EPA hazardous waste	No data	
OHM/TADS	No data	
DOT/UN/NA/IMCO shipping	UN 2810	DOT 2001
HSDB	336	HSDB 2001
NCI	No data	

CAS = Chemical Abstracts Services; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Material/Technical Assistance Data system; RTECS = Registry of Toxic Effects of Chemical Substances

Table 4-2. Typical Composition of Mustard Gas (H) from an Old Chemical Munition

Compound	CAS No.	GC/MS peak area percent
Mustard gas	505-60-2	62.2
Bis(2-chloroethyl) disulfide	1002-41-1	10.9
1,4-Dithiane	505-29-3	3.2
Bis(2-chloroethyl) trisulfide	19149-77-0	9.6
1,2-Bis(2-chloroethylthio)ethane	3563-36-8	2.6
1,2,3-Trithiolane	—	2.4
1,4-Thioxane	15980-15-1	0.1
1,2,5-Trithiepane	6576-93-8	0.9
1,2,3,4-Tetrathiane	—	1.4
1,2-Dichloroethane	107-06-2	3.2
HD Tetrasulfide	—	0.6
Tetrachloroethene	127-18-4	0.3
Sulfur	7704-34-9	0.5
Other	—	1.3

GC/MS = gas chromatography/mass spectrometry

Source: Rosenblatt et al. 1996

Table 4-3. Typical Composition of Mustard Gas (HD) in 1-Ton Storage Containers (Aberdeen, Maryland)

Compound	CAS No.	Mole percent
Mustard gas	505-60-2	91.38
Q sulfonium	30843-67-5	6.08
2-Chloroethyl 4-chlorobutyl sulfide	114811-35-7	0.86
1,4-Dithiane	505-29-3	0.81
1,2-Dichloroethane	107-06-2	0.35
Bis-3-chloropropyl sulfide	22535-54-2	0.18
2-Chloropropyl 3'-chloropropyl sulfide	—	0.18
2-Chloroethyl 3-chloropropyl sulfide	71784-01-5	0.14
1-Chloropropyl 2-chloroethyl sulfide	—	0.02
1,4-Thioxane	15980-15-1	<0.01

Source: NRC 1999

Table 4-4. Physical and Chemical Properties of Mustard Gas

Property	Information	Reference
Molecular weight	159.08	Munro et al. 1999
Color	Clear/pale yellow, black if impure	Munro et al. 1999
Physical state	Oily liquid	Munro et al. 1999
Melting point	13–14 EC	Munro et al. 1999
Boiling point	215–217 EC	Munro et al. 1999
	217.5 EC	Rosenblatt et al. 1996
Density:		
	1.338 at 13 EC	Budavari et al. 1996
	1.27 at 20 EC	Munro et al. 1999
	1.2685 at 25 EC	Rosenblatt et al. 1996
Odor	Weak, sweet, agreeable odor	Budavari et al. 1996
Odor threshold:		
Water	No data	
Air	0.6 mg/m^3	Bowden 1943
Solubility:		
Water	920 mg/L at 22 EC	Munro et al. 1999; Rosenblatt et al. 1996
	684 mg/L at 25 EC	Chemfate 2001
Organic solvent(s)	Soluble in alcohol, ether, acetone, benzene, miscible with petroleum ether	HSDB 2001
	Soluble in fat solvents and other common organic solvents	IARC 1975
Partition coefficients:		
Log K_{ow}	2.03	Chemfate 2001
	1.37	Munro et al. 1999; Rosenblatt et al. 1996
Log K_{oc}	2.07	Chemfate 2001
	2.12	Munro et al. 1999
Vapor pressure		
at 22 EC	0.082 mmHg	Rosenblatt et al. 1996
at 25 EC	0.1059 mmHg	Rosenblatt et al. 1996

Table 4-4. Physical and Chemical Properties of Mustard Gas *(continued)*

Property	Information	Reference
Henry's law constant	$2.57x10^{-5}$ atm-m^3/mol	Sage and Howard 1989
	$2.1x10^{-5}$ atm-m^3/mol	Munro et al. 1999
	$1.87x10^{-5}$ atm-m^3/mol	Rosenblatt et al. 1996
Autoignition temperature	No data	
Flashpoint	221 EF	Sax 1989
Conversion factors:	No data	
Explosive limits	No data	

5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.1 PRODUCTION

Mustard gas is a synthetic organic compound. It was first manufactured in 1822 by the action of ethene on sulfur monochloride or dichloride. Since then, the methods of manufacture have been refined although they have not been changed substantially. Three main processes have been used. The Germans produced mustard gas using the Meyer process, which involved treating ethylene with hypochlorous acid followed by sodium sulfide, yielding β,β'-dihydroxy-methyl sulfide. This was heated in turn with hydrochloric acid and produced mustard gas. In the United States, it was formerly made using the Levenstein process in which ethylene was reacted with sulphur monochloride at 30–35 EC. Mustard gas produced by this process contains 62–64% distilled mustard gas (or HD) (Munro et al. 1999). This process produces a complex mixture that includes constituents that are more toxic than mustard gas itself (Rosenblatt et al. 1975). The most recent process used in the United States involved the formation of bis-(2-hydroxyethyl)-thioether from ethylene oxide and hydrogen sulfide; this was then reacted with hydrochloric acid to form mustard gas (Franke 1967; IARC 1975; Rosenblatt et al. 1975). Mustard gas produced by this process contains 89% HD (Munro et al. 1999).

Mustard gas was manufactured in large quantities during World Wars I and II, but has not been manufactured on an industrial basis in the United States since 1968 (NRC 1994). Stockpiles of mustard gas are stored in 1-ton containers at Anniston Army Depot in Alabama, Umatilla Depot Activity in Oregon, Pine Bluff Arsenal in Arkansas, Tooele Army Depot in Utah, and Aberdeen Proving Ground in Maryland. Mustard gas is also stored as nonstockpile chemical materials in various containers and munitions at Pueblo Depot Activity in Colorado and Blue Grass Army Depot in Kentucky. Stockpiles of mustard gas are also located at the U.S. territory of Johnston Atoll in the North Pacific Ocean. These locations are illustrated in Figure 5-1 (Munro et al. 1999; NRC 1996). Mustard gas is probably still being made for laboratory experiments on a small scale.

5.2 IMPORT/EXPORT

Mustard gas is not imported into or exported from the United States.

Figure 5-1. Locations of Mustard Gas Storage Sites in the United States

Source: NRC 1996

5.3 USE

The principal use of mustard gas was as a vesicant chemical warfare agent. The Germans first used it against the British during World War I during the battle of Flanders, near Ypres, Belgium, in 1917 (Franke 1967; Rosenblatt et al. 1975). It was used by the Allies in 1918 and by the Italians in Ethiopia in 1936. It has also been used recently in the Iran-Iraq War in 1984–1988 and by Iraq against its Kurdish minority in Halabjah in 1988 (Black et al. 1993b; Budiansky 1984).

Attempts have been made to use mustard gas as an antineoplastic agent, although this has not met with much success due to its high toxicity. A similar product, nitrogen mustard, has been successfully employed as an anticancer agent (IARC 1975). Mustard gas has provided a useful model in biological studies concerning the behavior of alkylating agents (IARC 1975).

5.4 DISPOSAL

For the past several decades, the United States has stored its stockpile of mustard gas at seven Army facilities under a policy of total containment (Colburn 1978). The total quantity of mustard gas (i.e., H, HD, and HT) in the original stockpile was 17,358 tons (34,716,945 pounds) (DOA 2000). The stockpile consists of both munitions and 1-ton containers of bulk agent (see Table 5-1; DOA 2000; NRC 1994). In addition to mustard gas, munitions may contain energetics (e.g., explosives and propellents). Public Law 99-145 (as amended by PL 100-456) and PL 104-484 (October 23, 1992) requires the Army to destroy the U.S. stockpile of all lethal unitary chemical agents and munitions by December 31, 2004 (DOA 1988; NRC 1994). As part of the Chemical Stockpile Disposal Program (CSDP) mandated by Congress, the Army currently uses the "baseline system" for destruction of munitions and bulk agents containing mustard gas (NRC 1994).

The "baseline system" consists of several steps: (1) storage, transportation, and unloading of munitions and containers, (2) disassembly and draining, (3) agent destruction, (4) energetics destruction, (5) metal parts decontamination, and (6) dunnage (i.e., other contaminated materials) disposal (NRC 1994). Currently, munitions are stored and monitored in vented igloos; bulk containers are stored in the open or in monitored warehouses. The munitions or bulk mustard gas are transported to the on-site disposal facility and unloaded. Munitions are disassembled, drained of mustard gas, and separated into streams of bulk liquid agent, metal parts, energetics, and dunnage, all of which contain different amounts of mustard

Table 5-1. Original Stockpile Quantities of Mustard Gas as Munitions and Bulk Agent[a]

Chemical munitions or bulk agent	APG	ANAD	BAD	JAP	PBA	PUDA	TEAD	UMDA
HD								
105-mm projectile		68,500		140		1,138,760	5,860	
155-mm projectile		206,420	181,260	66,340		3,504,780		
4.2-inch mortar		452,160		116,294		460,340		
M60 projectile				261,960				
Ton container	3,249,740	185,080		116,294	188,400		11,383,420	
H								
155-mm projectile							639,540	
Ton container								4,679,040
HT								
4.2-inch mortar		1,064,600				118,220	363,020	
Ton container					6,249,100			
Total	3,249,740	1,976,760	181,260	578,705	6,437,500	5,222,100	12,391,840	4,679,040
Percent of total mustard gas stockpile	9.4	5.7	0.5	1.7	18.5	15.0	35.7	13.5

[a]Quantities of agent reported in pounds. Original stockpile quantities reflect amounts before the onset of Chemical Stockpile Disposal Program. Up-to-date information about stockpile quantities is available at http://www-pmcd.apgea.army.mil/.

ANAD = Anniston Army Depot, Alabama; APG = Aberdeen Proving Ground, Maryland; BAD = Blue Grass Army Depot, Kentucky; H = undistilled mustard gas; HD = distilled mustard gas; HT = 60% mustard gas + 40% Agent T; JAP = Johnston Atoll, Pacific Ocean; PBA = Pine Bluff Arsenal, Arkansas; PUDA = Pueblo Depot Activity, Colorado; TEAD = Tooele Depot, Utah; UMDA = Umatilla Depot Activity, Oregon

Source: DOA 2000

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gas. Liquid agent from drained munitions and bulk containers are fed into a primary incinerator preheated to an operating temperature of 2,700 EF (1,480 EC). Exhaust gases from the primary incinerator are fed into a secondary incinerator at a temperature of 2,200 EF (1,200 EC) for 2 seconds, after which 99.9999% of the agent is destroyed (DOA 2000). The gaseous effluents then flow into pollution abatement system before release into the atmosphere. Energetic materials are burned in a counterflow rotary kiln and then heated on a discharge conveyor at 1,000 EF (540 EC); the solid waste produced is nonhazardous and may be shipped for land disposal. Discharged gases pass through a secondary incinerator and a pollution abatement system, and then are released to the atmosphere. Metal parts are heated to 1,000 EF for 15 minutes in a fuel-fired metal parts furnace; the heat-treated metal parts are then released as scrap metal. Gases discharged pass through a secondary incinerator and a pollution abatement system, and then are released to the atmosphere. Dunnage generated during the entire process may be either incinerated (with pollution abatement) or shipped for land disposal as hazardous waste. At all steps, monitoring for chemical agents is performed to detect concentrations of agent well below those that present an immediate threat to personnel or the surrounding population. There are no measurable mustard gas effluents leaving the baseline system facilities under normal operating conditions (MacNaughton 2001). At present, Johnston Atoll is the first site to destroy its portion of the chemical agent and munitions stockpile in the United States (Kosson 2000). Incineration of mustard gas is currently underway at Umatilla, Oregon; Tooele, Utah; Anniston, Alabama; and Pine Bluff, Arkansas (SWRI 2001). There are two more sites at Blue Grass, Kentucky and Pueblo, Colorado, which have assembled chemical munitions. Incineration using the baseline system is scheduled to begin at these sites before 2005 (NRC 1994).

To address growing public concern over incineration, Congress in 1992 directed the Army to evaluate alternative disposal methods that might be significant safer and more cost effective than the baseline system (NRC 1994). Two alternatives were accepted by the Army for further development: (1) stand-alone neutralization followed by incineration and (2) neutralization followed by bio-treatment (NRC 1996). Neutralization of mustard gas is achieved by hydrolysis with hot water (90 EC) and vigorous mixing. This process reduces the mustard gas concentrations to levels <200 ppb and selectively converts 90% of the mustard gas to thiodiglycol and hydrochloric acid (Currie et al. 1977; May 1998; NRC 1996). Once the reaction is complete, base (e.g., sodium hydroxide or lime) is added to neutralize the acid and adjust the pH of the hydrolysate (i.e., product of hydrolysis). The dilute processing of mustard gas and the addition of base after completion of the neutralization reaction are designed to minimize the production of unwanted by-products during reaction (NRC 1996). Hydrolysis has been used effectively

to detoxify over 700 tons of mustard gas located at a Canadian defense facility in Cornwell, Ontario (Currie et al. 1977). After hydrolysis, the hydrolysate can be either incinerated using the baseline system or bio-treated. Bio-treatment requires adjusting the pH of the hydrolysate to neutral by adding sodium bicarbonate buffer and some added nutrients. Bacteria oxidize thiodiglycol to carbon dioxide, water, and sulfate with high efficiency. During the actual process, approximately 0.8 g of cell mass (dry weight) will be produced for every 1 g of organic carbon removed from solution. The biomass is further oxidized through aerobic digestion, and then dried and disposed of at commercial water treatment facility. Any volatile organic compounds (VOCs) that are present are condensed and the resulting condensate removed by direct photodegradation and photo-oxidation by OH radicals. The treated bioresidue is then filtered, dried, and sent to landfill (May 1998; NRC 1996). The chemical hydrolysis system is currently being developed for use at Aberdeen, Maryland, where mustard gas is stored only as a bulk liquid in 1-ton containers (NRC 1994).

6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

Mustard gas has been identified in at least 3 of the 1,585 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2001). However, the number of sites evaluated for mustard gas is not known. The frequency of these sites can be seen in Figure 6-1. Of these sites, 3 are located within the United States.

Mustard gas is not a naturally occurring compound and its only application is in chemical warfare. The United States has not used it for this purpose since World War I and has not produced it since 1968. Chemical agents, such as mustard gas, are extremely hazardous materials, which is why they were used as weapons. The hazard is increased when the agent is contained in explosively configured munitions, an inherent feature of chemical weapons. Since chemical weapons no longer have any value as a military deterrent, Congress has mandated that all chemical agents and munitions be destroyed by the end of the year 2004 (NRC 1994). Mustard gas is known to be stored at seven Army bases (see Section 5.1) across the continental United States, some of which may also be NPL sites (DOA 1988). Persons working at or living near Army bases where this material is stored or destroyed are at a greater risk of exposure.

6.2 RELEASES TO THE ENVIRONMENT

During World War I when mustard gas was being used, the compound was released directly to the atmosphere in countries outside the United States. It does not occur naturally, and is no longer produced in the United States. Mustard gas, which was produced for military applications, is now being stored in military depots and storage facilities across the United States (see Section 5.1). Both mustard gas agent and munitions are currently being destroyed on site at these Army facilities. All chemical agents maintained in the Army stockpile are now at least 30 years old and some are more than 54 years old; none were manufactured after 1968 (NRC 1994). There have been almost 1,500 “leaking” munitions identified in the stockpile since 1982, some of which are leaking mustard gas. In September 1993, a 100-gallon spill from a 1-ton container of mustard gas was discovered at Tooele Army Depot, Utah. The leak, which occurred around a corroded plug, produced a 10-foot by 12-foot pool of mustard gas on the ground. Other leaks of mustard gas have been identified from chemical munitions (e.g., 155-mm projectiles) as recently as June, 2000 (DOA 2000). Environmental releases of mustard gas may

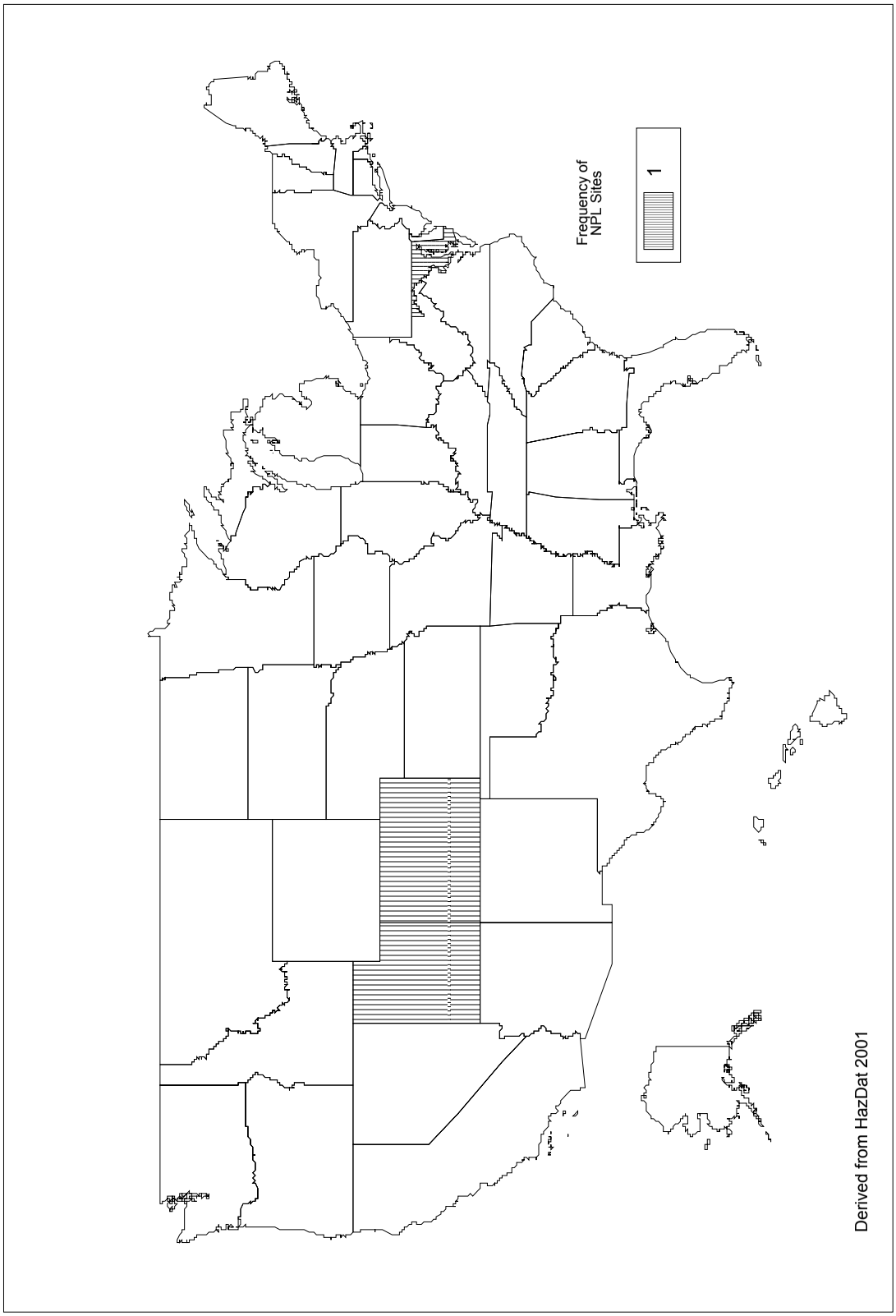


Figure 6-1. Frequency of NPL Sites with Mustard Gas Contamination

potentially occur near Army bases where this material is stored and destroyed; however, no releases of mustard gas beyond the confines of these facilities have been reported.

6.2.1 Air

Mustard gas may be released to air at sites across the United States where it is currently being destroyed by incineration. No known releases of mustard gas to the atmosphere by incineration have been reported (MacNaughton 2001).

6.2.2 Water

There are currently no known releases of mustard gas to water.

6.2.3 Soil

There are currently no known releases of mustard gas to soil.

6.3 ENVIRONMENTAL FATE

6.3.1 Transport and Partitioning

On the basis of its use during warfare and its physical/chemical properties, mustard gas should partition to and be transported in the atmosphere following release. The vapor pressure of mustard gas is moderate (0.11 mm Hg at 25 EC), but is high enough for mustard gas to be in air in the immediate vicinity of liquid droplets (DOA 1996).

On surface soil, Small (1984) reported that volatilization would be the main route of mustard gas loss. However, on moist surface soil, hydrolysis would be the main loss pathway. At 25 EC, mustard gas deposited on a surface soil evaporated within 30–50 hours (Munro et al. 1999). Meteorologic conditions such as temperature and wind will greatly affect the persistence of mustard gas on soil; with warmer temperatures and stronger winds, persistence of mustard gas decreases (Franke 1967). For example, mustard gas will vaporize 2–3 times faster at 20 EC than at 5 EC (Franke 1967). The freezing point of mustard gas is between 13 and 15 EC. In temperate regions, mustard gas should be a solid for half of the

year (Munro et al. 1999). The solidified mustard gas is less volatile, of lower water solubility, and is less reactive than liquified mustard gas. A study of persistence under winter conditions found that mustard gas could be detected after 2 weeks, but was below detection limits at 4 weeks (Franke 1967). When snowfall covered samples, high recoveries were demonstrated even after 4 weeks. This study also showed that persistence was affected by the size of droplets. Larger droplets of mustard gas increased both stability and recovery (Johnsen and Blanch 1984). Other factors that influence vaporization include pH, moisture content, porosity of the surface, and physical constituents of the soils (Rosenblatt et al. 1975). Because of its low solubility in water (920 mg/L) and ease of hydrolysis once dissolved, mustard gas is not transported through soil into groundwater (Munro et al. 1999).

In water, mustard gas will volatilize to air, hydrolyze, or remain unchanged. Without turbulence and at low temperatures, large qualitites of mustard gas would persist under water for long periods of time (Munro et al. 1999). Volatilization of mustard gas from water surfaces is expected to be moderate based upon a Henry's law constant of 2.1×10^{-5} atm•m^3/mol (DOA 1996). Using this Henry's law constant and an estimation method (Lyman et al. 1990), volatilization half-lives of mustard gas for a model river and model lake are 36 hours and 503 days, respectively. Hydrolysis of mustard gas may be slow because of its limited solubility and the fact that mustard gas freezes at 14 EC (see Section 6.3.2.2). Mustard gas is expected to sink to the bottom of the water column because it is denser than water (1.27 g/cm^3 at 20 EC; see Table 4-1).

Mustard gas does not bioconcentrate or biomagnify due to its reactivity. It is also unlikely that it is transported through the vascular systems of plants since it would almost surely undergo hydrolysis in the process (Rosenblatt et al. 1975).

6.3.2 Transformation and Degradation

6.3.2.1 Air

Mustard gas does not absorb ultraviolet (UV) radiation above 290 nm (Rewick et al. 1986); thus, photodegradation should not be an important fate process. The rate constant for the vapor-phase reaction of mustard with photochemically-produced hydroxyl radicals has been estimated as 7.82×10^{-12} cm^3/molecule-s at 25 EC using a structure estimation method (Meylan and Howard 1993). This corresponds to an atmospheric half-life of about 2.1 days at an atmospheric concentration of

$5x10^5$ hydroxyl radicals/m^3 (assumed average concentration in nonsmog conditions). Under smog conditions, reaction with nitrate radicals may be important.

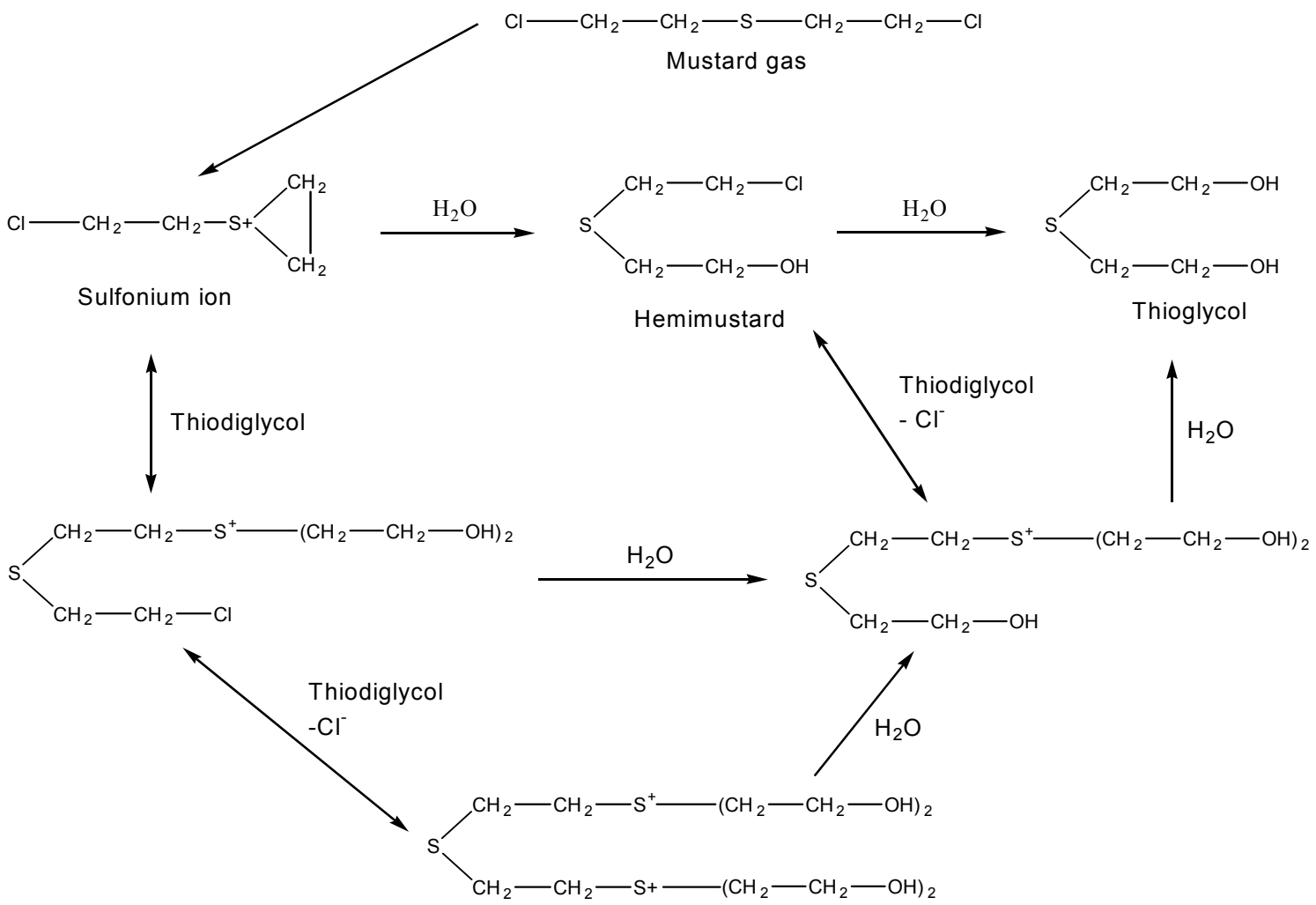
6.3.2.2 Water

Mustard gas rapidly hydrolyzes in water once dissolved (Bartlett and Swain 1949; Clark 1989; Rosenblatt et al. 1975; Small 1984; Stein 1946). Dissolved mustard gas has a hydrolysis half-life of 4–8 minutes at 25 EC in distilled water (Bartlett and Swain 1949). In several studies reviewed by Small (1984), the hydrolysis half-life (first-order rate) of dissolved mustard gas ranges from 158 minutes at 0.6 EC to ~1.5 minutes at 40 EC . However, because mustard gas is relatively insoluble (920 mg/L in water; Rosenblatt et al. 1996), hydrolysis is limited by its slow rate of solution (i.e., $6.77x10^{-8}$ g/cm^2 s at 10 EC). Hydrolysis of mustard gas is also retarded by polymerization of mustard gas, which forms a film at the liquid-liquid interface (Rosenblatt et al. 1996). This film interferes with the transfer of mustard gas into water and inhibits hydrolysis of the bulk agent. Thus, bulk mustard gas may persist in water for up to several years (Small 1984). Water-soluble organic solvents such as acetone and ethanol permit greater concentrations of mustard gas to solubilize in water so as to facilitate hydrolysis (Clark 1989). For example, when a small amount of acetone (e.g., 5% solution in water) was used to dissolve mustard gas in water at 25 EC, the hydrolysis half-life was 9.0 minutes (first-order rate constant=0.00129 s^{-1}).

The hydrolysis products of mustard gas are primarily mustard chlorohydrin, thiodiglycol, and hydrochloric acid; others include intermediates such as cyclic sulfonium salts (Rosenblatt et al. 1975, 1996). The hydrolysis of mustard chlorohydrin is somewhat faster than the hydrolysis of mustard gas. Consequently, mustard chlorohydrin does not accumulate to high concentrations. Conditions involving relatively small quantities of water give rise to higher concentrations of the cyclic sulfonium salt intermediates, which are rather toxic. Hydrolysis pathways of mustard gas in the environment are illustrated in Figure 6-2.

Sufficient levels of chlorine in the water (e.g., salt water) will inhibit the forward hydrolysis reaction; hydrolysis is decreased by a factor of 2.5 in salt water over fresh water (Clark 1989; Rosenblatt et al. 1975, 1996). Chloride ions react with the cyclic sulfonium intermediates to reform mustard gas. Impurities found in mustard gas (e.g., the polysulfide) might slow the dissolution of the agent, and if they dissolve in water, they will react more slowly with water than mustard gas (Rosenblatt et al. 1996). One

Figure 6-2. Primary Hydrolysis Pathways of Mustard Gas in the Environment



Source: Munro et al. 1999

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impurity, 1,2-bis(2-chloroethylthio)ethane, is about 5 times as vesicant as mustard gas itself; others, such as 1,8-dichloro-3-oxa-6-thiaoctane, are probably about as toxic as mustard gas.

Oxidation of mustard gas is also known to occur. Reactions with hypochlorite, chlorine water, ozone, and hydrogen peroxide yield mustard sulfoxide, which is extremely stable to hydrolysis and slightly toxic. Further oxidation under more severe conditions forms mustard sulfone, a relatively nontoxic compound. However, in weakly alkaline solution, mustard sulfone is dehydrochlorinated to divinyl sulfone, which is highly toxic (Clark 1989; Price and Bullitt 1947; Rosenblatt 1975).

6.3.2.3 Sediment and Soil

There is very little information on the transformation and degradation of mustard gas in soil. When mustard gas is released to soil, it remains relatively stable (Small 1984). It is known to degrade faster in alkaline soils (Franke 1967). If the soil was extremely wet, hydrolysis of mustard gas might occur to an appreciable extent (see Section 6.3.2.2). If mustard gas droplets are considerably below the soil surface, mustard gas can persist for several years (Munro et al. 1999; Watson and Griffin 1992). For example, mustard gas has been known persist for weeks to decades in military testing areas and land dumps where large quantities have been deposited underground. Mustard gas can be biodegraded in soil via the thioether oxidation pathway, forming bis-(2-chloroethyl)-sulfoxide and corresponding sulfone (U.S. Army Dugway Proving Ground 1985). Mustard gas can also be biodegraded via reductive dehalogenation and dehydrohalogenation, although these pathways are predicted to be slow.

6.3.2.4 Other Media

No information was found in the literature regarding transformation and degradation reactions in other media.

6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

6.4.1 Air

No information was found in the literature regarding environmental concentrations of mustard gas in the ambient atmosphere. However, ambient concentrations of mustard gas are expected to be zero except

near military facilities where former production occurred or where current disposal may be in progress. During World War I, when mustard gas was used, the average and maximum atmospheric concentrations in the combat zones were estimated at 3 and 5 ppm, respectively (IARC 1975).

6.4.2 Water

No information was found in the literature regarding environmental concentrations of mustard gas in surface water or groundwater.

6.4.3 Sediment and Soil

No information was found in the available literature regarding current soil concentrations of mustard gas. For some time after World War I, much of the French soil in the region of battle lines was contaminated, although it is unlikely to have persisted to the present day (IARC 1975). If any mustard gas still exists, it would be present only as pockets of liquid, perhaps dissolved in discarded oil, or absorbed on an inert anhydrous soil medium (Rosenblatt et al. 1975). Before 1945, mustard gas was produced at the Rocky Mountain Arsenal in Colorado. However, only traces of mustard gas have been found in soil samples at 3 or 4 locations out of 15,000 sampled during the recent clean-up of this site (Cohn 1999). Soils in Fort McClellan, Alabama are highly polluted with mustard gas and its many impurities (Dacre 1994). However, no additional information was found that quantifies the level of contamination at this site.

6.4.4 Other Environmental Media

Normal urinary levels of thiodiglycol, a hydrolysis product of mustard gas, are <1 ng/mL, but levels up to *ca.* 16 ng/mL are found in blood (Black and Read 1991). The source of this low backgrounds level is unknown, but sulfur-rich foods in the diet may be one possibility. No other information was found in the available literature regarding concentrations of mustard gas in environmental media.

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

The general population in the United States is not exposed to mustard gas since it is found only at seven Army bases that store it (Munro et al. 1999). Populations located near Army storage sites have a potentially greater risk of exposure. The entire stockpile of mustard gas agent and munitions are

presently being destroyed onsite at these facilities. Therefore, the risk of exposure to mustard gas from an accidental spill is decreasing. In countries where it was released during warfare, it is possible that conditions have been favorable to allow small quantities to persist. Unknown quantities of mustard gas were also released at some U.S. Army bases for training, research and development, and testing purposes. Small quantities may persist at these bases. Populations in these areas are at higher risk than those in areas that were never contaminated. Occupational exposure is limited to soldiers in combat where mustard gas is released or those involved in the storage and destruction of the compound. Construction workers may become exposed at Army bases where mustard gas was previously released and may persist in the soil or in an excavated munitions dump. The most recent report of its use is from the Iran-Iraq War in the 1980s when it was detected in the urine of some soldiers (Vycudilik 1985).

6.6 EXPOSURES OF CHILDREN

This section focuses on exposures from conception to maturity at 18 years in humans. Differences from adults in susceptibility to hazardous substances are discussed in 3.7 Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

Children in the United States are not likely to be exposed to mustard gas since it is found only at seven Army bases that store it (Munro et al. 1999), and access to the stored material is highly restricted.

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Since the U.S. supply of mustard gas is found in only at seven storage facilities (Munro et al. 1999), the potential for high exposures is limited to these areas and their surrounding communities. Exposure at or near Army storage facilities may occur if the munitions or storage containers explode or leak. However,

the stockpile of chemical weapons containing mustard gas is currently being destroyed, and thus the risk of accidental exposure to mustard gas is decreasing.

6.8 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of mustard gas is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of mustard gas.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.8.1 Identification of Data Needs

Physical and Chemical Properties. The physical and chemical properties of mustard gas are available (Tables 4-1 and 4-2). Experimental determination of properties for mustard gas such as log K_{ow}, log K_{oc}, and Henry's law constant values would be useful to determine its environmental fate.

Production, Import/Export, Use, Release, and Disposal. Since in 1968, mustard gas has not been produced, imported, or exported by the United States. Currently, the entire stockpile of mustard gas munitions and bulk agent are in the process of being destroyed. The destruction of the stockpile, mandated by Congress, is to be completed by December 31, 2004.

Environmental Fate. There is limited information on the environmental fate of mustard gas. It is known to vaporize and to degrade in water (Clark 1989; Rosenblatt et al. 1975; Stein 1946). Additional information on transport and transformation of mustard gas are needed to better characterize the environmental fate of the compound.

Bioavailability from Environmental Media. Mustard gas can be absorbed following inhalation (Drasch et al. 1987; Somani and Babu 1989) and dermal (Cullumbine 1946, 1947; Drasch et al. 1987; Nagy et al. 1946; Renshaw 1946) exposure from air and soil. This was its intended use and it is well studied (see Chapter 3).

Food Chain Bioaccumulation. No information was found regarding the bioconcentration of mustard gas by plants, animals, and aquatic organisms, or biomagnification in terrestrial or aquatic food chains. However, due to the toxicity and metabolism of mustard gas, it is unlikely that it will bioconcentrate or biomagnify.

Exposure Levels in Environmental Media. There are limited reports of mustard gas being detected in environmental media, at hazardous waste sites, Army chemical weapon stockpile facilities, or at other locations. Additional information is needed on media concentration levels near former and current facilities where it has been produced, stored, or destroyed. This information will be useful in predicting human exposure levels at these locations.

Exposure Levels in Humans. Mustard gas metabolites have been detected in the urine and blood of exposed humans after its use as a chemical weapon (see Chapter 7). For example, the compound thiodiglycol has been detected in the urine of soldiers after an acute exposure to mustard gas (Wils et al. 1985). However, the use of levels in urine or other biomarkers has not been reported in any other exposed populations. More sensitive methods of detection may be useful for assessment of chronic exposure to individuals working or living near facilities that store or destroy mustard gas.

Exposures of Children. Mustard gas has been detected in exposed children after its use as a chemical weapon (See Chapter 3) during the Iran-Iraq War (Momeni and Aminjavaheri 1994). More sensitive methods of detection for mustard gas may be useful for assessment of chronic exposure to children living near facilities that store or destroy mustard gas.

Child health data needs relating to susceptibility are discussed in 3.12.2 Identification of Data Needs: Children's Susceptibility.

Exposure Registries. There are no exposure registries for mustard gas. This compound is not currently one of the compounds for which a subregistry has been established in the National Exposure Registry. The compound will be considered in the future when chemical selection is made for subregistries to be established. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to the exposure to this compound.

6.8.2 Ongoing Studies

There are no ongoing studies for mustard gas focusing on the potential for human exposure (FEDRIP 2001).

7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring mustard gas, its metabolites, and other biomarkers of exposure and effect to mustard gas. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

7.1 BIOLOGICAL SAMPLES

The most common currently used method of analyzing for the presence of mustard gas and its metabolites in biological and environmental samples is gas chromatography/mass spectrometry (GC/MS). Prior to 1987, however, thin-layer chromatography (TLC) with a colorimetric detection system and gas chromatography with either flame ionization detector (FID), electron capture detector (ECD), or flame photometric detector (FPD) were the most frequently used. Sample preparation consists primarily of extraction with an organic solvent. Sodium chloride is sometimes added to improve sample stability and prevent mustard gas breakdown to thiodiglycol and other metabolites. Depending on the method used, and the possible interfering compounds present, further cleanup and preparative steps may be included. No specific EPA, NIOSH, or AOAC methods were found for this chemical. Table 7-1 summarizes several representative analytical methods for detecting mustard gas and its metabolites in biological samples.

Little information was found on the direct detection of mustard gas in biological tissues or fluids. However, in two cases of suspected exposure, sodium chloride was first added to the urine samples to stabilize any mustard gas that might be present. A semi-quantitative analysis by GC/MS detected low-ppb levels of mustard gas in these samples compared to none detected in a control sample of a definitely unexposed person (Vycudilik 1985, 1987). The detection limit of the procedure was in the low parts per billion range with recoveries of about 20%. Mustard gas has also been detected in the body

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Table 7-1. Analytical Methods for Determining Mustard Gas in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Saturate with sodium chloride; extract with diethyl ether; centrifuge; isolate organic phase and evaporate; redissolve in methylene chloride; clean up with silica gel; centrifuge; evaporate solvent layer; redissolve in methylene chloride	GC/MS (EI)	10 ng/µL injected	20%	Vycudilik 1985
Urine	Hydrolyze sample with helix pomatia (enzymatic hydrolysis); clean up on carbon column; add concentrated hydrochloric acid to convert thio-diglycol to mustard gas; headspace analysis® with collection on Tenax; thermally desorb (Thiodiglycol)	GC/MS	1 µg/L (1 ppb)	75%	Wils et al. 1988
Urine	Treat samples with acidic titanium trichloride; final residue dissolved in acetonitrile and toluene	GC-MS-MS	0.1 µg/L (0.1 ppb)	48–56%	Black and Read 1995b
Human fluids and tissues	Homogenize tissue; extract sample with dichloro-methane; centrifuge; remove dichloromethane layer and evaporate; redissolve in hexane; clean up on TLC; remove sample spots and complex with gold; extract with toluene	ET-AAS	1.1 mg/L (ppm, body fluids); 0.1 mg/kg (ppm, body tissues)	No data	Drasch et al. 1987

EI = electron impact; ET-AAS = electrothermal atomic adsorption spectroscopy; GC = gas chromatography; GC-MS-MS = gas chromatography-tandem mass spectroscopy; MS = mass spectroscopy; TLC = thin layer chromatography

tissues and fluids of an alleged victim (Drasch et al. 1987). In this analysis, abdominal fat samples were first qualitatively analyzed by GC/MS. A quantitative analysis of several more tissues and fluids by electrothermal atomic absorption spectroscopy (ET-AAS) detected mustard gas in blood and in all tissues examined. Detection limits were 1.1 mg/L in body fluids and 0.1 mg/kg in body tissues (low-ppm range).

Mustard gas is generally metabolized rapidly in biological systems. The primary method of analyzing for mustard gas exposure is by detecting the presence of its hydrolysis metabolites in biological fluids. GC/MS has been used for this purpose. The procedure involves conversion of the most common hydrolysis metabolite, thiodiglycol, to mustard gas by heating with concentrated hydrochloric acid (Wils et al. 1985, 1988). The detection limit for this procedure is in the low ppb range (about 1 µg/L) and with inclusion of deuterated thiodiglycol as an internal standard, recoveries of 75% are obtained (Wils et al. 1988). Unfortunately, thiodiglycol (and thiodiglycol sulphoxide) can exist in the urine of both exposed and nonexposed subjects; detection of thiodiglycol in human urine by this procedure at a concentration level of 10–100 µg/L does not prove mustard gas poisoning (Wils et al. 1985). Other methods using GC/MS have determined mustard gas in urine of exposed rats and guinea pigs by derivatisation of thiodiglycol with heptafluorobutyric anhydride (Jakubowski et al. 1990). Black and co-workers quantified thiodiglycol (and thiodiglycol sulphoxide) in urine of exposed humans using GC/MS after formation of bis(pentafluorobenzoyl) derivatives (Black and Read 1991, 1995a, 1995b; Black et al. 1992a, 1992b, 1994).

Another recent method for mustard gas detection in urine is gas chromatography-tandem mass spectrometry (GC-MS-MS) with selected-reaction monitoring. This method was applied to the analysis of urinary metabolites of mustard gas derived from hydrolysis (i.e., thiodiglycol and its sulfoxide) and the glutathione pathway after further metabolism involving the enzyme β-lyase (i.e., 1,1-sulphonylbis[2-(methylsulphinyl)ethane] and 1-methylsulphinyl-2-[2-(methylthio)ethylsulphonyl]ethane). The procedure involves treatment of samples with acidic titanium trichloride to reduce thiodiglycol sulfoxide to thiodiglycol and the two β-lyase metabolites to a single analyte, 1,1-suphonylbis[2-(2-methykthio)ethane]. The detection limit for this procedure is in the sub-ppb range (0.1 µg/L) for detection of β-lyase metabolites and in the ppb range (1–12 µg/L) for detection of thiodiglycol. Recoveries, determined in normal urine spiked with 1,1-suphonylbis[2-(2-methykthio)ethane] at a concentration of 1 µg/L, ranged from 48 to 56%. The advantage of this method is that β-lyase metabolites of mustard gas have not been observed in normal urine and this method provides an unequivocal biological marker of exposure to mustard gas (Black and Read 1995b).

Recently, the detection of DNA adducts formed by modification of DNA by mustard gas in blood offers a promising approach for retrospective detection of exposure. For example, Ludlum et al. (1994) detected a *N*7-guanine adduct of DNA using high performance liquid chromatography (HPLC) with fluorometric monitoring. In this study, the authors were able to detect one *N*7-guanine adduct in $3x10^5$ DNA nucleotides. Benschop and co-workers (Benschop et al. 1997; Fidder et al. 1996a) were able to confirm the exposure to mustard gas in samples taken in March 1988 from two Iranians. Exposure to mustard gas was verified by two independent methods based on immunochemical analysis of the *N*7-guanine adduct in DNA and GC/MS analysis of the *N*-terminal valine adduct in globin after a modified Edman degradation. The adduct levels found were considerably higher than the detection limit for the modified Edman procedure (i.e., 0.1 µM mustard gas), but just above the detection limit for the immunochemical assay (i.e., 0.07 µM mustard gas). In another study, Noort et al. (1996) described the use of liquid chromatography-tandem mass spectrometry (LC-MS-MS) to identify modified sites in human hemoglobin after *in vitro* exposure to mustard gas. They note that hemoglobin is efficiently alkylated by mustard gas leading to an increase in 104 m/z after hydrolysis. This method is based on cleavage of globin by trypsin and micro-LC-MS analysis of the digests.

7.2 ENVIRONMENTAL SAMPLES

Until recently, GC with FID, ECD, or FPD were the primary methods of analysis for mustard gas and its metabolites, with a colorimetric assay utilizing 4-(*p*-nitrobenzyl)pyridine also frequently used. GC/MS is more commonly used for detecting mustard gas and its metabolites in environmental samples. Table 7-2 presents a summary of several common analytical techniques used to analyze for mustard gas and its metabolites in environmental samples.

Separation by TLC, followed by detection with a 4-(*p*-nitrobenzyl) pyridine procedure has been used qualitatively and quantitatively to detect sulfur mustard in the presence of other vesicant mustards (Sass and Stutz 1981; Stutz and Sass 1969). This technique has proved useful in detecting mustard gas in a variety of complex matrices (water, soil, plants) and has a detection limit of 1 µg/sample spot (Sass and Stutz 1981). In addition to being relatively sensitive and selective, it can be scaled up for preparative work and down for small samples. This gives it continued usefulness despite the advent of more sophisticated GC/MS techniques.

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Table 7-2. Analytical Methods for Determining Mustard Gas in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collect in decalin solvent using double trap system	GC/ECD	0.2 ng/µL injected	99.5–101.5%	Casselman et al. 1973
Air	Collect in diethyl succinate using double trap system	GC/FPD	0.2 ng/µL injected	98–101%	Gibson et al. 1974
Air	Collection in Tenax GC in a glass tube; thermal desorption into GC	GC/FPD	10 ng/m^3	No data	Fowler and Smith 1990
Water	Directly inject sample for thiodiglycol detection; extract with hexane and concentrate for detection of other compounds (metabolites)	GC/FID	50 µg/L (ppb)	No data	D'Agostino et al. 1989
		GC/MS (CI)	No data	No data	D'Agostino et al. 1989
		GC/FTIR	No data	No data	D'Agostino et al. 1989
Water or vapor	Extract with hexane	GC/ECD	160 µg/L (water); 1 µg/L (vapor)	No data	Fisher et al. 1969
Standard solutions and vapors	Dissolve standard of known purity in hexane or chloroform (mustard gas and metabolites)	GC/ECD GC/FPD	About 160 µg/L (solution); about 1 µg/L (vapor)	No data	Sass and Steger 1982
Soil	Extract with chloroform; sonicate (mustard gas and metabolites)	GC/MS (CI)	5–10 ng/injection	No data	D'Agostino and Provost 1988b
	No data	GC/MS (EI)	No data		Vycudilik 1985
Mustard gas hydrolysate	Extract with hexane; concentrate	GC/FID	No data	No data	D'Agostino and Provost 1988a

CI = chemical ionization; ECD = electron capture detector; EI = electron impact; FID = flame ionization detector; FPD = flame photometric detector; FTIR = Fourier transform infrared spectroscopy; GC = gas chromatography; MS = mass spectrometry

GC with either FID, ECD, or FPD was the most common technique of the 1970s and early 1980s for determining the presence of mustard gas and its metabolites, and is still frequently used. It has been used to detect mustard gas in air by passing air through a solvent trap. Aliquots of the solvent are directly injected into the gas chromatograph to detect mustard gas (Casselman et al. 1973; Gibson et al. 1974). With both ECD and FPD, recoveries were near 100%, and the detection limit was 0.2 ng/µL injected. Advantages of both were speed, simplicity, and reliability. However, the solvent producing the best results with ECD required ice-bath cooling to prevent solvent and mustard gas loss (Casselman et al. 1973). The solvent used with FPD had the advantage of allowing room temperature analysis (Gibson et al. 1974).

GC was used to detect mustard gas in water (Fisher et al. 1969) and soil (D'Agostino and Provost 1988a). Using GC/ECD, a minimum detection limit (quantifiable) of 160 µg/L (ppb) for aqueous solutions and 1 µg/L for vapor was obtained. The method used was simple, selective, and precise. The authors proposed that with appropriate sample preparation, it could be used for a variety of media including soil and biological media (Fisher et al. 1969). Mustard gas and metabolites were detected in soil by GC/FID (D'Agostino and Provost 1988a), who also analyzed a hydrolysate remaining from the destruction of munitions grade mustard, but no details on accuracy, precision, or sensitivity are given. A comparison of the various detectors used to analyze for mustard gas and its metabolites was conducted and showed ECD to be the most sensitive for detecting mustard gas in a mixture of mustard compounds, followed by FPD, and FID (Sass and Steger 1982). The detection limit using ECD and FPD was in the mid-ppb range (about 160 µg/L) for solutions and in the low-ppb range (about 1 µg/L) for vapors. Beck et al. (2001) found that GC-FPD provided a rapid and sensitive method for analysis of thiodiglycol (TDG) in soil extracts with a detection limit of 1.1 µg/g soil. Pressurized liquid extraction (PLE) with methanol- water (9:1) proved to be the most efficient solvent for TDG extraction with recoveries ranging from 12 to 89% of added TDG for various soil types.

GC/MS has been used to analyze for the presence of mustard gas and its metabolites (D'Agostino and Provost 1988b; D'Agostino et al. 1989; Munavalli and Jakubowski 1989; Vycudilik 1985). Tests with pure substances have supported the sensitivity, selectivity, and reliability of this technique, and analysis of pure samples have proved its usefulness. Inclusion of deuterated thiodiglycol as an internal standard increases the accuracy of GC/MS and makes the technique quantitative as opposed to simply semi-quantitative. Both chemical ionization (CI) and electron impact (EI) have been used to detect mustard gas and its characteristic metabolites in samples. Detection of specific mustard metabolites is important

in determining mustard gas exposure since the chemical can degrade rapidly under certain environmental conditions. Testing of several EI and CI techniques showed that MS was a sensitive, reliable, and precise detection method for mustard gas (Ali-Mattila et al. 1983). Later studies on sample mixtures of vesicant mustards and degradation products, as well as on water and soil samples supported this (D'Agostino and Provost 1988b, 1992; D'Agostino et al. 1989; Munavalli and Jakubowski 1989; Vycudilik 1985). For example, D'Agostino and Provost (1992) used GC/MS for verification of mustard gas and its hydrolysis products in soil. They used sequential hexane and dichloromethane extraction followed by trimethylsilyl derivatization and achieved total recoveries in the 50–90% range for most soil types. Wils et al. (1992) used GC/MS to analyze mustard gas in rubber and paint samples in combination with diesel fuel and aromatic white spirit as a background. Mustard gas was isolated by extraction with methylene chloride or by dynamic headspace analysis at elevated temperatures. Recoveries of mustard gas in rubber and paint ranged from 57 to 86%. Black et al. (1993b) analyzed soil, bomb casing, and sheep wool samples associated with a chemical weapons agent (CWA) incident (obtained from a Kurdish village in the Northern part of Iraq in 1988) by GC/MS using headspace analysis, solvent extraction, and thermal desorption methods. Using this technique, the presence of mustard gas and 21 related compounds were successfully confirmed in these samples.

Mustard gas vapor is typically determined in air by bubbling an air sample through a liquid solvent and analyzing the solvent for absorbed mustard by colorimetry or by gas chromatography. However, the colorimetric technique lacks specificity and the solvent entrapment sampling technique possesses a number of drawbacks such as limited analyte-trapping efficiency, high detection limits, and degradation of the analyte (Fowler and Smith 1990). Rapid and accurate methodologies for detection of mustard gas have been developed for use during the demilitarization of mustard stockpiles at U.S. storage sites. These procedures are based largely on the Depot Area Air Monitoring System (DAAMS) technology (Smith et al. 1982). DAAMS procedures have undergone extensive Precision and Accuracy studies (Smith and Fowler 1985) and are methods of choice in current and future demilitarization sites. The sampling and analysis process for DAAMS consist of (1) collection of the airborne sample on the sorbent (Tenax GC) in a glass tube, (2) transfer to a glass tube containing smaller amounts of the same sorbent using an external thermal desorber, and (3) thermal desorption in a specially-modified injection port of a gas chromatograph and subsequent analysis using a flame photometric detector (Fowler and Smith 1990; Posner 1991). The detection limit of mustard gas vapor in air by these procedures is about 10 ng/m^3 (or 1.5 parts per trillion).

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of mustard gas is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of mustard gas.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Available information indicates that sensitive, selective, and reliable methods for determining biomarkers of exposure exist for mustard gas and its metabolites (Black and Read 1991, 1995a, 1995b; Black et al. 1992a, 1992b, 1993b, 1994; Kientz 1998; Ludlum et al. 1994). Available studies emphasize detection and quanitification of the compound and its metabolites. Further studies that attempt to quantify levels in exposed and unexposed populations might be useful in assessing the risk associated with mustard gas and its metabolites.

Effect. As discussed, sensitive, selective, and reliable methods, exist for detecting mustard gas and its metabolites in biological tissues and fluids. Available studies do not emphasize quantifying the levels of these compounds and associating the amounts found with specific biomarkers of effect. Further studies associating specific levels in fluids and tissues with known effects could be useful in assessing the risk associated with mustard gas and its metabolites.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Sensitive, selective, and reliable methods exist for detecting mustard gas and its metabolites in air (Casselman et al. 1973; Gibson et al. 1974), water (Fisher et al. 1969; Sass and Stutz 1981; Stutz and Sass 1969), and soil (D'Agostino and Provost 1988a; Sass and Stutz 1981; Stutz and Sass 1969). No information was obtained on detection in other environmental media. The available methods emphasize qualitative and quantitative detection. Further studies to improve the detection of mustard gas and its metabolites could aid in assessing the potential risk of mustard gas in the environment, especially near hazardous waste facilities and Army storage facilities.

7.3.2 Ongoing Studies

No ongoing studies were located.

8. REGULATIONS AND ADVISORIES

Mustard gas is on the list of chemicals appearing in "Toxic Chemicals Subject to Section 313 of the Emergency Planning and Community Right-to-Know Act of 1986" (EPA 1987). Available information on regulations and standards is presented in Table 8-1.

ATSDR has derived an acute inhalation MRL of 0.0002 mg/m^3 based on a LOAEL of 21.3 mg/m^3 for respiratory effects in mice that were exposed for 1 hour. The LOAEL was duration-adjusted to a 24-hour exposure period, dosimetrically adjusted for humans, and an uncertainty factor of 300 [10 for use of a LOAEL, 3 for extrapolation from animals to humans using a dosimetric adjustment, and 10 for human variability] and a modifying factor of 3 [for proximity to serious effects (28% body weight loss at 16.9 mg/m^3)] were applied to the LOAEL to derive the MRL (see Appendix A for details).

ATSDR has not derived intermediate or chronic inhalation MRLs for mustard gas because quantitative data were not available to determine NOAELs or LOAELs.

ATSDR has derived an acute oral MRL of 0.0005 mg/kg/day (0.5 µg/kg/day) based on a LOAEL of 0.5 mg/kg/day for inflamed mesenteric lymph nodes in rats that were exposed for 10 days. An uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability) was applied to the LOAEL to derive the MRL.

An intermediate-duration oral MRL of 0.00002 mg/kg/day (0.02 µg/kg/day) was based on a time-weighted average LOAEL of 0.02 mg/kg/day (see Appendix A for details) for gastrointestinal effects in rats that were exposed for a 21-week period. An uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability) was applied to the LOAEL to derive the MRL.

ATSDR has not derived a chronic oral MRL for mustard gas because quantitative data were not available to determine NOAELs or LOAELs.

The Centers for Disease Control and Prevention (CDC) has proposed mustard gas airborne exposure limits (AELs) for agent workers and the general population to provide adequate protection during the limited time of potential exposure prior to the completion of the Chemical Stockpile Demilitarization

Program (CDC 1988). For agent workers, the AEL proposed for mustard gas as an 8-hour time-weighted average (TWA) is 0.003 mg/m^3. For the general population, a mustard gas 72-hour TWA AEL of 0.0001 mg/m^3 and a ceiling value indicating a maximum exposure concentration at any time, for any duration, of 0.003 mg/m^3 have been proposed. The CDC noted that uncertainties in the quantitative risk assessment methodology employed to derive the proposed limits preclude their use as acceptable precise limits for mustard gas exposure.

The National Toxicology Program (2001) reports that mustard gas is known to be a human carcinogen. The International Agency for Research on Cancer (IARC) has classified mustard gas as carcinogenic to humans (Group 1), based on sufficient evidence for carcinogenicity to humans and limited evidence for carcinogenicity to animals (IARC 2001).

On October 17, 1986, the President signed into law the Superfund Amendments and Reauthorization Act of 1986 (SARA). This act amended the Comprehensive Environmental Response, Compensation and Liability Act of 1980 (CERCLA), commonly known as "Superfund". The Emergency Planning and Community Right-to-Know Act of 1986 (EPCRA) was included under Title III of SARA.

EPA has established a reportable quantity (RQ) for mustard gas of 500 pounds under the CERCLA section 103, codified at 40 CFR part 302, in addition to the requirements of 40 CFR part 355, and regulates it as a hazardous constituent of waste under the Resource Conservation and Recovery Act (RCRA, 40 CFR 261).

EPA regulates mustard gas under the Superfund Amendments and Reauthorization Act (SARA), subjecting it to reporting requirements. Emergency response plans are required under SARA if the threshold planning quantity (TPQ) of 500 pounds is exceeded.

Under EPCRA, release of mustard gas must be reported according to EPA toxic chemical release reporting regulations (40 CFR 372.65).

Mustard gas is included as a constituent regulated under the groundwater protection standards for inactive uranium processing sites (40 CFR 192).

OSHA regulates mustard gas under the Hazard Communication Standard and as a chemical hazard in laboratories.

The Department of Veterans Affairs regulates compensation based on chronic effects of exposure to mustard gas (38 CFR 3.316).

Table 8-1. Regulations and Guidelines Applicable to Mustard Gas

Agency	Description	Information	References
INTERNATIONAL			
Guidelines:			
IARC	Carcinogenicity classification	Group 1[a]	IARC 2001
NATIONAL			
Regulations and Guidelines:			
a. Air			
ACGIH	TLV-TWA	No data	
OSHA	PEL (TWA)	No data	
NIOSH	REL	No data	
b. Water		No data	
c. Food		No data	
d. Other			
BEA	Chemical Weapons Convention requirements—schedules of chemicals		BEA 2001 15CFR745
DOS	International traffic in arms regulations—United States munitions list; chemical agents		DOS 2001 22CFR121.7
DOT	Hazardous materials table		DOT 2001 49CFR172.101
EPA	CERCLA—reportable quantity	500 pounds	EPA 2001a 40CFR355 Appendix B
	Groundwater protection standards at inactive uranium processing sites		EPA 2001b 40CFR192, Appendix I
	SARA—extremely hazardous substance (TPQ)	500 pounds	EPA 2001a 40CFR355, Appendix B
	RCRA—identification and listing as hazardous waste		EPA 2001c 40CFR261, Appendix VIII

Table 8-1. Regulations and Guidelines Applicable to Mustard Gas *(continued)*

Agency	Description	Information	References
NATIONAL *(cont.)*			
EPA	Toxic chemical release reporting; Community Right-to-Know		EPA 2001d 40CFR372.65
	Effective date	01/01/87	
NTP	Carcinogenic classification	Known to be a human carcinogen	NTP 2001
OSHA	Meets criteria for proposed medical records rule		OSHA 1982
VA	Claims based on chronic effects of exposure		VA 2001 38CFR3.316
STATE			
Regulations and Guidelines:			
a. Air:			
Colorado	Air contaminant emission notice		BNA 2001
Connecticut	HAP		BNA 2001
Maryland	Toxic air pollutant—known human carcinogen		BNA 2001
b. Water			
New York	Water regulation TPQ RQ	 1 pound 1 pound	BNA 2001
c. Food		No data	
d. Other			
Alabama	Identification and listing of hazardous waste		BNA 2001
Arkansas	Identification and listing of hazardous waste		BNA 2001
California	Chemical known to cause cancer or reproductive toxicity—initial appearance of chemical on list	02/27/87	BNA 2001
	Hazardous substance list		BNA 2001
Colorado	Identification and listing of hazardous waste		BNA 2001

Table 8-1. Regulations and Guidelines Applicable to Mustard Gas *(continued)*

Agency	Description	Information	References
STATE *(cont.)*			
District of Columbia	Identification and listing of hazardous waste		BNA 2001
Delaware	Reportable quantity	1 pound	BNA 2001
Florida	Toxic substances in the workplace		BNA 2001
Georgia	Regulated substance and soil concentration that trigger notification		BNA 2001
Illinois	Identification and listing of hazardous waste		BNA 2001
Kentucky	Extremely hazardous substance—TPQ	500 pounds	BNA 2001
	Identification and listing of hazardous waste		BNA 2001
Louisiana	Hazardous waste		BNA 2001
Maine	Identification and listing of hazardous waste		BNA 2001
Massachusetts	Containers adequately labeled pursuant to federal law		BNA 2001
	Oil and hazardous material list		BNA 2001
Maryland	Identification and listing of hazardous waste		BNA 2001
Michigan	Identification and listing of hazardous waste		BNA 2001
Minnesota	Hazardous constituent		BNA 2001
Nebraska	Hazardous constituent		BNA 2001
New Jersey	Discharge of oil and other hazardous substances		BNA 2001
North Dakota	Identification and listing of hazardous waste		BNA 2001
Ohio	Toxic release inventory rules		BNA 2001
Oregon	Toxic use reduction and hazardous reduction regulations		BNA 2001

Table 8-1. Regulations and Guidelines Applicable to Mustard Gas *(continued)*

Agency	Description	Information	References
STATE *(cont.)*			
South Carolina	Identification and listing of hazardous waste		BNA 2001
Tennessee	Identification and listing of hazardous waste		BNA 2001
Vermont	Hazardous waste management regulation		BNA 2001
Washington	Dangerous waste regulations		BNA 2001
Wisconsin	Identification and listing of hazardous waste		BNA 2001
Wyoming	Identification and listing of hazardous waste		BNA 2001

[a]Group 1: Carcinogenic to humans

ACGIH = American Conference of Governmental Industrial Hygienists; BEA = Bureau of Export Administration; BNA = Bureau of National Affairs; CERCLA = Comprehensive Environmental Response, Compensation, and Liability Act; CFR = Code of Federal Regulations; DOS = Department of State; DOT = Department of Transportation; EPA = Environmental Protection Agency; HAP = hazardous air pollutant; IARC = International Agency for Research on Cancer; NIOSH = National Institute of Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; RCRA = Resource Conservation and Recovery Act; REL = relative exposure limit; RQ = reportable quantity; SARA = Superfund Amendments and Reauthorization Act; TPQ = threshold planning quantity; TLV = threshold limit values; TWA = time-weighted average; VA = Department of Veteran Affairs

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10. GLOSSARY

Absorption—The taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by a sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD)—Usually defined as the lower confidence limit on the dose that produces a specified magnitude of changes in a specified adverse response. For example, a BMD_{10} would be the dose at the 95% lower confidence limit on a 10% response, and the benchmark response (BMR) would be 10%. The BMD is determined by modeling the dose response curve in the region of the dose response relationship where biologically observable data are feasible.

Benchmark Dose Model—A statistical dose-response model applied to either experimental toxicological or epidemiological data to calculate a BMD.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility.

Cancer Effect Level (CEL)—The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study which examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-controlled study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without outcome.

Case Report—Describes a single individual with a particular disease or exposure. These may suggest some potential topics for scientific research but are not actual research studies.

Case Series—Describes the experience of a small number of individuals with the same disease or exposure. These may suggest potential topics for scientific research but are not actual research studies.

Ceiling Value—A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure—Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome. At least one exposed group is compared to one unexposed group.

Cross-sectional Study—A type of epidemiological study of a group or groups which examines the relationship between exposure and outcome to a chemical or to chemicals at one point in time.

Data Needs—Substance-specific informational needs that if met would reduce the uncertainties of human health assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the adverse effects.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurs. The terms, as used here, include malformations and variations, altered growth, and *in utero* death.

Environmental Protection Agency (EPA) Health Advisory—An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Epidemiology—Refers to the investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one half of a quantity of a chemical from the body or environmental media.

Immediately Dangerous to Life or Health (IDLH)—The maximum environmental concentration of a contaminant from which one could escape within 30 minutes without any escape-impairing symptoms or irreversible health effects.

Incidence—The ratio of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

Immunologic Toxicity—The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

Immunological Effects—Functional changes in the immune response.

In Vitro—Isolated from the living organism and artificially maintained, as in a test tube.

In Vivo—Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air which has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{LO})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical which has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a minimal risk level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—State of being diseased; morbidity rate is the incidence or prevalence of disease in a specific population.

Mortality—Death; mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations. A mutation is a change in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a chemical.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) which represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An odds ratio of greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed.

Organophosphate or Organophosphorus Compound—A phosphorus containing organic compound and especially a pesticide that acts by inhibiting cholinesterase.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) allowable exposure level in workplace air averaged over an 8-hour shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests.

Pharmacokinetics—The science of quantitatively predicting the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments which, in general, do not represent real, identifiable anatomic regions of the body whereby the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically-based dose-response model which quantitatively describes the relationship between target tissue dose and toxic end points. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—Comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information: tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates and, possibly membrane permeabilities. The models also utilize biochemical information such as air/blood partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which the pertinent observations are made on events occurring after the start of the study. A group is followed over time.

q_1*—The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually µg/L for water, mg/kg/day for food, and µg/m^3 for air).

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentrations for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation reference concentration is for continuous inhalation exposures and is appropriately expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the no-observed-adverse-effect level (NOAEL-from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior,

fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a chemical.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, or an inborn or inherited characteristic, that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed.

Short-Term Exposure Limit (STEL)—The American Conference of Governmental Industrial Hygienists (ACGIH) maximum concentration to which workers can be exposed for up to 15 minute continually. No more than four excursions are allowed per day, and there must be at least 60 minute between exposure periods. The daily Threshold Limit Value - Time Weighted Average (TLV-TWA) may not be exceeded.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a Time Weighted Average (TWA), as a Short-Term Exposure Limit (STEL), or as a ceiling limit (CL).

Time-Weighted Average (TWA)—An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

Toxic Dose$_{(50)}$ (TD$_{50}$)—A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Toxicokinetic—The study of the absorption, distribution and elimination of toxic compounds in the living organism.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL) or Reference Dose (RfD) or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-

observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of one can be used; however a reduced UF of three may be used on a case-by-case basis, three being the approximate logarithmic average of 10 and 1.

Xenobiotic—Any chemical that is foreign to the biological system.

APPENDIX A
ATSDR MINIMAL RISK LEVEL AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as a hundredfold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology, expert panel peer reviews, and agencywide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road, Mailstop E-29, Atlanta, Georgia 30333.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEETS

Chemical name: Mustard gas [bis(2-chlorethyl) sulfide]
CAS number(s): 505-60-2
Date: August 21, 2001
Profile status: Third Draft
Route: [X] Inhalation [Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Key to figure: 3
Species: Mice

Minimal Risk Level: 0.0002 [ ] mg/kg/day [ ] ppm [X] mg/m^3

Reference: Vijayaraghavan R. 1997. Modifications of breathing pattern induced by inhaled sulphur mustard in mice. Arch Toxicol 71:157-164.

Experimental design: Groups of female Swiss albino mice (four mice/group, 24–26 g) were administered 8.5, 16.9, 21.3, 26.8, 42.3, or 84.7 mg/m^3 of mustard gas (>99% purity, dissolved in acetone and nebulized) 1 time by inhalation (head only) for 1 hour. Negative and vehicle control groups (four mice/group) were exposed similarly to filtered air and acetone, respectively. Respiratory variables including frequency, tidal volume (VT), inspiratory time, expiratory time, time of brake, time of pause, and flow at 0.5 tidal volume were recorded using an automated system. Breathing patterns were measured up to 7 days after exposure.

Effects noted in study and corresponding doses: Deaths first occurred 6 days after exposure (incidence not reported). The estimated LC50 for 14 days of observation was 42.5 mg/m^3. Mice were unaffected by exposure to acetone as a vehicle control (5760 mg/m^3 estimated acetone concentration, 0.04% of RD50). During exposures, at all mustard gas concentrations administered, mice exhibited sensory irritation, 15-20 min after the start, characterized by a pause between inspiration and expiration. There was no pulmonary irritation during exposure. Clinical observations revealed swollen heads and closed eye lids in animals at concentrations greater than 21.3 mg/m^3 (incidence data not reported). The authors reported that the animals were lethargic (concentrations not specified). Decreases in body weight began 24 hours after exposure, were concentration-related, and achieved statistical significance ($p<0.05$) at concentrations of 16.9 mg/m^3 or higher. At 7 days post-exposure, body weights were decreased from pre-treatment values by 2%, 13%, 28%, 25%, 32%, and 34% in the control, 8.5, 16.9, 21.3, 26.8, and 42.3 mg/m^3 exposure groups. Respiratory frequency, which decreased to a slower steady state after 30 min of exposure, was depressed by approximately 20% at the lowest concentration to a maximum of 64% for concentrations $42.3 mg/m^3. Following exposure, the depression in respiratory frequency was related to both concentration and post-exposure time. While sensory irritation was reversible, delayed effects of mustard gas were indicated by a significant reduction in respiratory frequency beginning 48 h after exposure at concentrations of 21.3 mg/m^3 and higher. No pauses between respiratory cycles were measured at any exposure level, from which the authors concluded a lack of pulmonary irritation and toxic effects limited to the upper respiratory tract. Airflow limitation was evidenced by a lengthening of time of expiration and a decreased respiratory rate. Airflow limitation was thought to occur due to the effect of mustard gas on the tracheal secretory cells. ATSDR considers the respiratory system the critical target for toxic effects, with a LOAEL of 21.3 mg/m^3 identified for delayed respiratory effects beginning 48 hours after exposure. While significant reductions in body weight occurred at $16.9 mg/m^3 in the principal study (Vijayaraghavan, 1997), in two other studies in mice (Pant and Vijayaraghavan 1999) and guinea pigs (Allon, et al. 1993) weight reductions of only 14% were observed at 85 and 125 mg/m^3, respectively, at

similar post-exposure times. Therefore, ATSDR considers the magnitude of the weight reduction at 16.9 mg/m^3 (28%) an outlier in the database.

Dose and end point used for MRL derivation: ATSDR considers the concentration of 21.3 mg/m^3 a LOAEL for delayed respiratory effects. A significant reduction in respiratory frequency was observed beginning 48 h after exposure at concentrations of 21.3 mg/m^3 and higher.

[ ] NOAEL [X] LOAEL

Uncertainty factors used in MRL derivation:
[X] 10 for use of a LOAEL
[X] 3 for extrapolation from animals to humans using a dosimetric adjustment
[X] 10 for human variability
[X] 3 Modifying factor for proximity to serious effects (28% body weight reduction)

Was a conversion factor used from ppm in food or water to a mg/body weight dose? NA

Was a conversion used from intermittent to continuous exposure? Yes
$LOAEL_{[ADJ]} = 21.3\ mg/m^3 \times (1h / 24h) = 0.89\ mg/m^3$

If an inhalation study in animals, list conversion factors used in determining human equivalent dose:
Because mustard gas is water soluble and induces respiratory effects, the chemical is defined as a category 1 gas according to U.S. EPA (1994). The human equivalent LOAEL for a category 1 gas, $LOAEL_{[HEC]}$, is the product of the $LOAEL_{[ADJ]}$ and the ratio of the regional gas dose in animal species to that of humans for the region (r) of interest for the toxic effect ($RGDR_r$) (EPA 1994). For extrathoracic (ET) effects, $RGDR_{ET}$ is approximated by $(V_E/SA_{ET})_A/(V_E/SA_{ET})_H$, where V_E = minute volume (mL/min) and SA_{ET} is the surface area of the extrathoracic region (cm^2) (EPA 1994). For tracheobronchial (TB) effects, $RGDR_{TB}$ is approximated by $[(V_E/SA_{TB})_A \times \exp(SA_{ET}/V_E)_A]/[(V_E/SA_{TB})_H \times \exp(SA_{ET}/V_E)_H]$(EPA 1994). The extrathoracic surface areas for humans and mice are 200 and 3 cm^2, respectively, and the tracheobronchial surface areas, 3200 and 3.5 cm^2, respectively (EPA 1994). The minute volumes for humans and mice are 13800 and 48.8, respectively (EPA 1987c). Accordingly, $RGDR_{ET}$ and $RGDR_{TB}$ are 0.24 and 3.0, respectively. While the authors thought that airflow limitation, evidenced as a decreased respiratory rate, was due to the effect of mustard gas on the tracheal secretory cells, it is possible that the decreased respiratory rate was due to effects sensed by receptive structures in the extrathoracic region. Therefore, the more conservative $RGDR_{ET}$ of 0.24 was used to derive the MRL. $LOAEL_{[HEC]} = LOAEL_{[ADJ]} \times RGDR_{ET} = 0.89\ mg/m^3 \times 0.24 = 0.21\ mg/m^3$. Application of an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans using a dosimetric adjustment, 10 for human variability) and a modifying factor of 3 for proximity to serious effects (28% body weight loss at 16.9 mg/m^3) yields an acute inhalation MRL of 0.0002 mg/m^3.

Other additional studies or pertinent information that lend support to this MRL:
In a study in which female albino mice were administered 21.3, 42.3 or 84.6 mg/m^3 of mustard gas (> 99% purity, dissolved in acetone and nebulized) one time by inhalation (head only) for 1 h, there were significant time- and dose-dependent increases in the excretion of urinary uric acid following exposure (6 - 48 h post-exposure at mid- and high-doses, 24 - 48 h at low-dose) (Kumar and Vijayaraghavan 1998). In the low- and mid-dose groups, urinary uric acid levels showed a decline from peak levels by 7 days post-exposure, whereas levels in the high-dose group continued to increase. Blood uric acid also increased in a dose- and time-dependent manner (6 - 48 h post-exposure at high-dose, 24 - 48 h at low- and mid-doses). A plateau or decline was noted in blood uric acid levels at 7 days post-exposure in all

dose groups. These results suggest reversible kidney effects in the 21 - 42 mg/m^3 range and more serious effects at 85 mg/m^3, which would be protected against by the acute inhalation MRL derived from the principal study (Vijayaraghavan, 1997) LOAEL of 21.3 mg/m^3.

Agency Contact (Chemical Manager): Zemoria A. Rosemond

MINIMAL RISK LEVEL (MRL) WORKSHEETS

Chemical name: Mustard gas [bis(2-chlorethyl) sulfide]
CAS number(s): 505-60-2
Date: July 8, 2001
Profile status: Third Draft
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Key to figure: 3, 4
Species: Rat

Minimal Risk Level: 0.5 [X] µg/kg/day [ ] ppm [ ] mg/m^3

Reference: DOA. 1987b. Teratology studies on lewisite and sulfur mustard agents: Effects of sulfur mustard in rats and rabbits. Fort Detrick, MD: U.S. Army Medical Research and Development Command, U.S. Department of Army. AD-A187 495.

Experimental design: Mustard gas (95.9–96.1% purity) in sesame oil was administered by intragastric intubation to mated Sprague-Dawley female rats (10–11 weeks old) on gestation days 6 through 15 (10 days). The administered doses were 0, 0.5, 1.0, or 2.0 mg/kg/day and there were 25–27 animals/dose group, of which 20–26/dose group were pregnant. All animals were observed for clinical signs of toxicity prior to and following administration of mustard gas. Treated rats were weighed on gestation days 0 and 6–15 (exposure days), and on day 20. Necropsy was performed on all rats found dead or in moribund condition. Scheduled necropsy was performed on gestation day 20. Blood samples were collected from maternal animals for hematocrit measurement prior to sacrifice. The animals were examined for gross lesions of major organ systems. The numbers of corpora lutea, implantation sites, resorptions, and live and dead fetuses were determined. Uterine weights were recorded. Live fetuses were removed, weighed, sexed, and examined for gross, soft tissue, and skeletal anomalies.

Effects noted in study and corresponding doses: There were no treatment-related deaths. In rats, a significant dose-related decrease in maternal body weight was observed by gestation day 12 at 0.5 mg/kg/day (4.1–6.6%) and by gestation day 9 in the 1.0 (4.7–9.1%) and 2.0 (6.5–16.0%) mg/kg/day groups. Extragestation weight gain was significantly reduced at $0.5 mg/kg/day with dose-related reductions of 25, 38, and 57% at 0.5, 1.0, and 2.0 mg/kg/day compared to controls. A significantly decreased (16%) gravid uteri weight was measured at the highest dose. Maternal hematocrit values were statistically significantly reduced by 5.4% at 1.0 and 2.0 mg/kg/day. Gastric mucosa inflammation was observed in 2/30 (6.7%) rats at 2.0 mg/kg/day, but not in any of the lower dose or control groups. A significant incidence of inflamed mesenteric lymph nodes was found at $0.5 mg/kg/day; the incidences were 0/34 controls, and 11/25 (44%), 16/25 (64%), and 7/30 (57%) rats at 0.5, 1.0, and 2.0 mg/kg/day, respectively.

Fetal body weight was significantly decreased (6–7%) from controls in litters exposed to doses of 1.0 and 2.0 mg/kg/day; no clear dose-relation was evident. The sex ratio (percent males) was significantly lower than control at the highest dose (46.2 vs. 51.0%). Placental weight was also significantly reduced (8.4%) at the highest dose. Supernumerary ribs were found in 9/299 (3%) fetuses of one litter in the highest dose group, while this anomaly was not found in any of the fetuses in the lower dose or control groups. The incidence of reduced ossification of the vertebrae and/or sternebrae in all treated groups was significantly higher than controls when individual pup data were compared, but not with litter comparisons, 42/272 (15%) in controls, 51/229 (22%) at 0.5 mg/kg/day, 76/315 (24%) at 1.0 mg/kg/day, and

72/299 (24%) at 2.0 mg/kg/day. All fetal effects in rats occurred at doses that also produced maternal toxicity.

Dose and end point used for MRL derivation: The lowest dose tested in rats, 0.5 mg/kg/day is a LOAEL for inflamed mesenteric lymph nodes in the dams and reduced ossification in the fetuses.

[ ] NOAEL [X] LOAEL

Uncertainty factors used in MRL derivation:

[X] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? NA

Was a conversion used from intermittent to continuous exposure? NA

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: NA

Other additional studies or pertinent information that lend support to this MRL: In support of the critical effect, there is some evidence in humans to indicate that mustard gas affects the lymph system. Discoloration of the lymph nodes in the axillary, inguinal, and mesenteric glands were noted in autopsies of victims of the World War II Bari Harbor incident, during which mustard gas was released in to the air and water (Alexander 1947). The spleen also demonstrated evidences of gross pathology in 33 of 53 (62%) autopsies (Alexander 1947). In the majority of cases, the spleen was described as shrunken in size with pale color. Microscopically, only 2 of 32 spleens examined showed degeneration or necrosis; pyknosis and karyorrhexis of lymphocytes in some corpuscles were observed in one and slight necrosis of the malpighian follicle, was observed in the other. Additional studies in animals also revealed mustard gas-induced damage to the lymph system. Cameron et al. (1946), after observing damage to the cervical lymph nodes and lymphoid tissue throughout the body in rabbits and monkeys that had undergone tracheal cannulation and had been exposed to chamber concentrations of mustard gas ranging from 30 to 350 mg/m^3 (5–54 ppm), administered mustard gas to animal skin topically or by subcutaneous injection and observed identical changes to the lymph tissue, suggesting that lymphoid tissue damage may be due to systemic absorption. Mustard gas produced a significant dose-related decrease in the weight of peripheral lymph nodes (12–44%) when topically applied at single doses of 3.88, 7.75, or 15.5 mg/kg to the shaved backs of Balb/c mice (Venkateswaran et al. 1994). A significant decrease in the weight of mesenteric lymph nodes (18%) was noted at the highest dose. Incidence and severity of histological changes in the thymus and spleen were also dose-related. Spleen histopathology included hypocellularity, atrophy of the lymphoid follicles, degeneration of germinal centers, and red pulp infiltrated with macrophages. The cortex and medulla regions of the thymus showed atrophy and hypocellularity. A significant dose-related decrease in the cellularity of the spleen (24–45%) was measured. A dose-related decrease in the cellularity of the thymus was also found, and was significant at the mid- and high doses (36–42%). A significant dose-related reduction in spleen cell number was measured in female mice 7 days after intraperitoneal injection with mustard gas (23% at 5 mg/kg and 49% at 10 mg/kg) (Coutelier et al. 1991).

The principal study (DOA 1987b) identified the lowest LOAEL of 0.5 mg/kg/day for inflamed mesenteric lymph nodes in rats following acute administration of mustard gas. In range-finding experiments,

conducted prior the principal teratology study, in which rats were dosed with 0, 0.2, 0.4, 0.8, 1.6, 2.0, or 2.5 mg/kg/day (3–9 animals/dose group of which 2–7/dose group were pregnant), significant incidences of inflamed mesenteric lymph nodes occurred at $0.4 mg/kg/day (DOA 1987b). Also in support of the critical dose, another lymphoretic effect, enlarged Peyer's patches, was observed in rabbits at 0.5 g/kg/day in a range-finding study and at 0.4 g/kg/day in a teratology study (incidence data were not reported) (DOA 1987b).

Agency Contact (Chemical Manager): Zemoria A. Rosemond

MINIMAL RISK LEVEL (MRL) WORKSHEETS

Chemical name: Mustard gas [bis(2-chlorethyl) sulfide]
CAS number(s): 505-60-2
Date: July 8, 2001
Profile status: Third Draft
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Key to figure: 8
Species: Rat

Minimal Risk Level: 0.02 [X] µg/kg/day [ ] ppm [ ] mg/m^3

Reference: Sasser L., et al. 1996a. Two-generation reproduction study of sulfur mustard in rats. Repro Toxicol 10(4):311-319.

Experimental design: Mustard gas (97.3% purity) in sesame oil was administered intragastrically to groups of 8-week-old Sprague-Dawley rats (27 females and 20 males/group/generation) at doses of 0, 0.03, 0.1, or 0.4 mg/kg/day. Male and female rats were dosed 5 days/week for 13 week before mating and during a 2-week mating period. Males were dosed 5days/week during the 21-day gestation period. Females were dosed daily (7 days/week) throughout the 21-day gestation period and 4-5 days/week during the 21-day lactation period. Mated males were sacrificed at the birth of their pups, and dams, were sacrificed when their pups were weaned. Male and female F1 pups were treated with mustard gas until they were mated and the females became pregnant and gave birth. F1 males were sacrificed at the birth of their pups. The dosing of F1 dams continued until pup weaning, at which time, the study was terminated. Animals were weighed weekly. A complete gross necropsy was performed on all rats found dead or in moribund condition. Weights of the testis, prostate, epididymis, ovary, and uterus were recorded. Histopathological evaluations were performed on reproductive organs of the high dose group and control group of the F0 and F1 adults and on the forestomach of animals in all dose groups.

Effects noted in study and corresponding doses: There were no treatment-related deaths. The body weights of the F0 exposed rats were not significantly different from controls; however, the growth rate of the high-dose males tended to decline after about 7 weeks of exposure. Body weight gain was significantly lower ($p<0.05$) than control values in F1 rats of both sexes born to high-dose parents beginning 1 or 2 weeks after gavaging was started (approximately 20% for males and 15–24% for females). No significant dose-response in body weight occurred at the lower doses. Breeding and reproductive performance in F0 and F1 animals was not affected by treatment. The only statistically significant birth parameter difference was an increase in the sex ratio (fraction of males) of the high-dose F0 offspring. Although not significantly different, litter weights and number of pups per litter tended to decrease in both F1 and F2 animals at the highest exposure level. Except for a slight reduction in absolute ovary weight in high-dose F0 females, absolute and/or relative male and female reproductive organ weights were unaffected by treatment. Microscopic examination of the reproductive organs revealed no evidence treatment-related effects. Dose-related incidence and severity of lesions of the squamous epithelium of the forestomach characterized by cellular disorganization of the basilar layer, an apparent increase in mitotic activity of the basilar epithelial cells and thickening of the epithelial layer, occurred in both sexes of each treatment group. The incidence of hyperplasia (combined F0 and F1 males and females) was 0/94 controls, 69/94 (73%; 27 males/42 females) in the low-dose groups, 90/94 (96%; 39 males/51 females) in the mid-dose groups, and 94/94 in the high-dose groups. Benign neoplasms of

the forestomach (squamous papilloma) occurred in 0/94 controls, 0/94 in the low-dose groups, 8/94 (9%) in the mid-dose groups, and 10/94 (11%) in the high-dose groups.

Dose and end point used for MRL derivation:

The lowest dose tested, 0.03 mg/kg/day, is a LOAEL for gastric lesions.

[ ] NOAEL [X] LOAEL

Uncertainty factors used in MRL derivation:

[X] 10 for use of a LOAEL
[X] 10 for extrapolation from animals to humans
[X] 10 for human variability

Was a conversion factor used from ppm in food or water to a mg/body weight dose? NA

Was a conversion used from intermittent to continuous exposure? A time-weighted average (TWA) daily dose was calculated as follows. Females were dosed during the lactation period while males were not. Female rats were gavaged 5days/week for 15 weeks (75 days), total dose=2.25 mg/kg (75 days x 0.03 mg/kg/day); daily for 3 gestation weeks (21 days), total dose=0.63 mg/kg; and 4days/week for 3 lactation weeks (12days), total dose=0.36 mg/kg. The cumulative dose for females over the 21-week period is 3.24 mg/kg (2.25+0.63+0.36 mg/kg). Dividing the cumulative dose of 3.24 by 147 days (21 weeks) yields a TWA dose of 0.02 mg/kg/day. For males, the TWA dose would be 0.03 mg/kg/day x (5 days/7 days) =0.02 mg/kg/day.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: NA

Other additional studies or pertinent information that lend support to this MRL: Injury to the gastric mucosa is a portal-of-entry toxic effect that is consistent with the vesicant properties of mustard gas following oral exposure. In support of the critical effect, gastrointestinal effects have been reported in humans following combat exposure to mustard gas, in mustard gas testing volunteers, and in mustard gas factory workers. In all of these cases, exposure was likely by multiple routes including inhalation, oral, and dermal. In 19 of 53 (36%) victims of the World War II Bari Harbor incident autopsied, stomach irritation and inflammation were documented. The lesions varied from simple hyperemia to focal loss of epithelium, necrosis, and ulceration (Alexander 1947). In a review of the clinical manifestations of mustard gas exposure in the Iran-Iraq war victims, Pierard et al. (1990) reported that endoscopy frequently revealed acute gastritis. Incidences of gastrointestinal effects of nausea (64%), vomiting (43%), and bleeding (14%) were reported in a group of 14 children and teenagers following exposure to mustard gas from air bombs during the Iran-Iraq war (Momeni and Aminjavaheri 1994). Gastrointestinal neoplasms were reported in Japanese mustard gas factory workers who were involved with the production of chemical agents during World War II (Yamakido et al. 1985). Mustard gas testing volunteers who were wearing respirators and who were exposed to unspecified levels of mustard gas vapors and liquids had skin burns, but also complained of nausea, vomiting, anorexia, abdominal pain, diarrhea, headache, and lassitude (Sinclair 1948). These signs could have been primary effects of the mustard gas on the rapidly dividing cells of the gastrointestinal epithelium, secondary effects from the skin burns, or psychological effects not related to the mustard gas exposure at all.

In addition to the principal study, Sasser et al. (1996a), similar gastric effects edema, hemorrhage or sloughing of the mucosa, and ulceration) have been identified in rabbits following 14-day exposures at $0.4 mg/kg/day (DOA 1987b), in rats following 10-day exposures at $2.0 mg/kg/day (DOA 1987b), and in rats following 13-week exposures at $0.1 mg/kg/day (Sasser et al. 1996b). Regarding the relevance of the toxic effects to humans lacking a forestomach, tissue damage at the point of contact would be expected by a vesicant and direct alkylating agent such as mustard gas, regardless of the location in the gastrointestinal tract.

Agency Contact (Chemical Manager): Zemoria A. Rosemond

APPENDIX B

USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Relevance to Public Health

This chapter provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions.

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The chapter covers end points in the same order they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter. If data are located in the scientific literature, a table of genotoxicity information is included.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal risk levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, we have derived minimal risk levels (MRLs) for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action; but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans. They should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses for lifetime exposure (RfDs).

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest NOAEL that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the LSE Tables.

Chapter 3

Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables (3-1, 3-2, and 3-3) and figures (3-1 and 3-2) are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, minimal risk levels (MRLs) to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of No-Observed-Adverse-Effect Levels (NOAELs), Lowest-Observed-Adverse-Effect Levels (LOAELs), or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See LSE Table 3-1

(1) Route of Exposure One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. When sufficient data exists, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Table 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not therefore have all five of the tables and figures.

(2) Exposure Period Three exposure periods - acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more) are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the 2 "18r" data points in Figure 3-1).

(5) Species The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration The duration of the study and the weekly and daily exposure regimen are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to 1,1,2,2-tetrachloroethane via inhalation for 6 hours per day, 5 days per week, for 3 weeks. For a more complete review of the dosing regimen refer to the appropriate sections of the text or the original reference paper, i.e., Nitschke et al. 1981.

(7) System This column further defines the systemic effects. These systems include: respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, 1 systemic effect (respiratory) was investigated.

(8) NOAEL A No-Observed-Adverse-Effect Level (NOAEL) is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL A Lowest-Observed-Adverse-Effect Level (LOAEL) is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference The complete reference citation is given in Chapter 9 of the profile.

(11) CEL A Cancer Effect Level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Figure 3-1

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period The same exposure periods appear as in the LSE table. In this example, health effects observed within the intermediate and chronic exposure periods are illustrated.

(14) Health Effect These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL In this example, 18r NOAEL is the critical end point for which an intermediate inhalation exposure MRL is based. As you can see from the LSE figure key, the open-circle symbol indicates to a NOAEL for the test species-rat. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the Table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL Key number 38r is 1 of 3 studies for which Cancer Effect Levels were derived. The diamond symbol refers to a Cancer Effect Level for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) Key to LSE Figure The Key explains the abbreviations and symbols used in the figure.

SAMPLE

1 6

Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
2 6 INTERMEDIATE EXPOSURE							
	5	6	7	8	9		10
3 6 Systemic	9	9	9	9	9		9
4 6 18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
CHRONIC EXPOSURE							
					11		
Cancer					9		
38	Rat	18 mo 5 d/wk 7 hr/d			20	(CEL, multiple organs)	Wong et al. 1982
39	Rat	89–104 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, nasal tumors)	NTP 1982
40	Mouse	79–103 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, hemangiosarcomas)	NTP 1982

[a] The number corresponds to entries in Figure 3-1.

12 6 [b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5 x 10^{-3} ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

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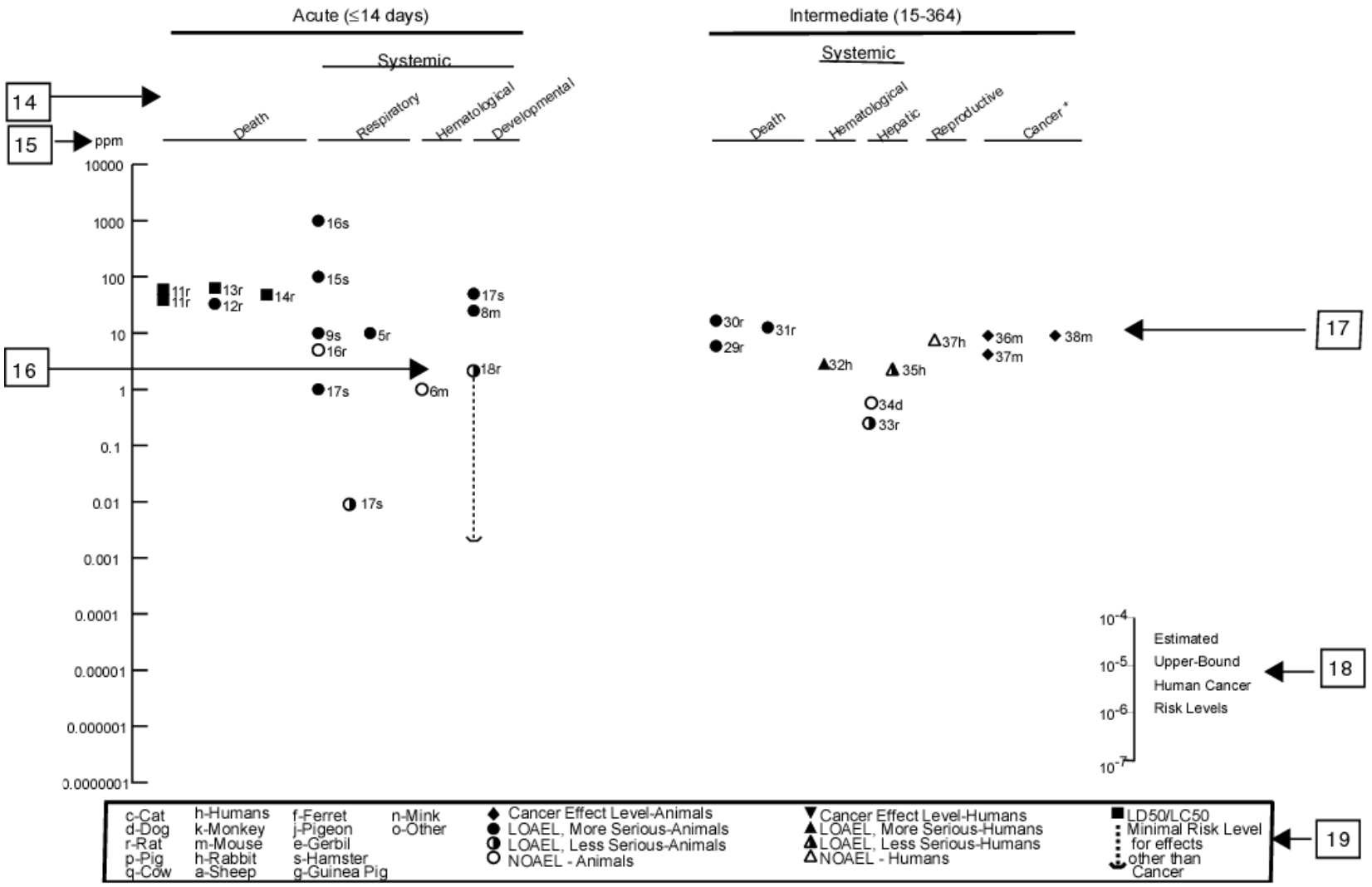
SAMPLE

13 → Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation



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APPENDIX C

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolism, and Excretion
AFID	alkali flame ionization detector
AFOSH	Air Force Office of Safety and Health
AML	acute myeloid leukemia
AOAC	Association of Official Analytical Chemists
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BAT	Best Available Technology
BCF	bioconcentration factor
BEI	Biological Exposure Index
BSC	Board of Scientific Counselors
C	Centigrade
CAA	Clean Air Act
CAG	Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	Cancer Effect Level
CELDS	Computer-Environmental Legislative Data System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CL	ceiling limit value
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CNS	central nervous system
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
d	day
Derm	dermal
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor
DOT	Department of Transportation
DOT/UN/ NA/IMCO	Department of Transportation/United Nations/ North America/International Maritime Dangerous Goods Code
DWEL	Drinking Water Exposure Level
ECD	electron capture detection
ECG/EKG	electrocardiogram
EEG	electroencephalogram

EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F_1	first-filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FPD	flame photometric detection
fpm	feet per minute
ft	foot
FR	*Federal Register*
g	gram
GC	gas chromatography
Gd	gestational day
gen	generation
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
hr	hour
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IDLH	Immediately Dangerous to Life and Health
IARC	International Agency for Research on Cancer
ILO	International Labor Organization
in	inch
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{Lo}	lethal concentration, low
LC_{50}	lethal concentration, 50% kill
LD_{Lo}	lethal dose, low
LD_{50}	lethal dose, 50% kill
LT_{50}	lethal time, 50% kill
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
m	meter
MA	*trans,trans*-muconic acid
MAL	Maximum Allowable Level
mCi	millicurie
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
mg	milligram
min	minute
mL	milliliter
mm	millimeter

mm Hg	millimeters of mercury
mmol	millimole
mo	month
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NATICH	National Air Toxics Information Clearinghouse
NATO	North Atlantic Treaty Organization
NCE	normochromatic erythrocytes
NCI	National Cancer Institute
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
NFPA	National Fire Protection Association
ng	nanogram
NLM	National Library of Medicine
nm	nanometer
NHANES	National Health and Nutrition Examination Survey
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPD	nitrogen phosphorus detection
NPDES	National Pollutant Discharge Elimination System
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NSPS	New Source Performance Standards
NTIS	National Technical Information Service
NTP	National Toxicology Program
ODW	Office of Drinking Water, EPA
OERR	Office of Emergency and Remedial Response, EPA
OHM/TADS	Oil and Hazardous Materials/Technical Assistance Data System
OPP	Office of Pesticide Programs, EPA
OPPTS	Office of Prevention, Pesticides and Toxic Substances, EPA
OPPT	Office of Pollution Prevention and Toxics, EPA
OSHA	Occupational Safety and Health Administration
OSW	Office of Solid Waste, EPA
OTS	Office of Toxic Substances
OW	Office of Water
OWRS	Office of Water Regulations and Standards, EPA
PAH	Polycyclic Aromatic Hydrocarbon
PBPD	Physiologically Based Pharmacodynamic
PBPK	Physiologically Based Pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
PID	photo ionization detector
pg	picogram

pmol	picomole
PHS	Public Health Service
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSNS	Pretreatment Standards for New Sources
REL	recommended exposure level/limit
RfC	Reference Concentration
RfD	Reference Dose
RNA	ribonucleic acid
RTECS	Registry of Toxic Effects of Chemical Substances
RQ	Reportable Quantity
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
sec	second
SIC	Standard Industrial Classification
SIM	selected ion monitoring
SMCL	Secondary Maximum Contaminant Level
SMR	standard mortality ratio
SNARL	Suggested No Adverse Response Level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short term exposure limit
STORET	Storage and Retrieval
TD_{50}	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TOC	Total Organic Compound
TPQ	Threshold Planning Quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TRI	Toxics Release Inventory
TWA	time-weighted average
U.S.	United States
UF	uncertainty factor
VOC	Volatile Organic Compound
yr	year
WHO	World Health Organization
wk	week

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
µm	micrometer
µg	microgram

q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result

APPENDIX D

INDEX